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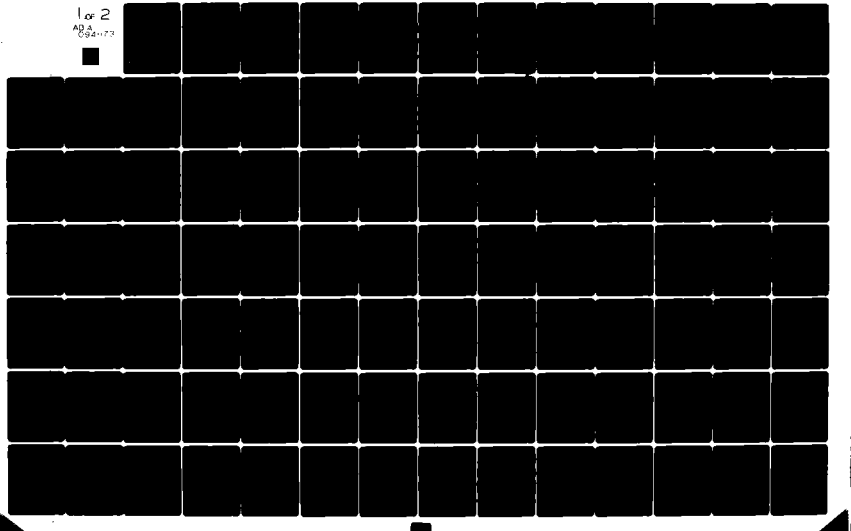

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SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
		AD A094873

4. TITLE (and Subtitle)
Specialized Genetic Recombination Systems in Bacteria: Their Involvement in Gene Expression and Evolution.

5. TYPE OF REPORT & PERIOD COVERED

6. PERFORMING ORG. REPORT NUMBER

7. AUTHOR(s)
D.J. Kopecko

15

8. CONTRACT OR GRANT NUMBER(s)

9. PERFORMING ORGANIZATION NAME AND ADDRESS
Department of Bacterial Immunology SGRD-UWF-H
Division of CD&I, WRAIR, WRAMC
Washington, DC 20012

10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS

11. CONTROLLING OFFICE NAME AND ADDRESS
U.S. Army Medical Research & Development Command
Fort Detrick, Frederick, Md 21701

12. REPORT DATE

13. NUMBER OF PAGES
99

14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)
Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington, D.C. 20012

15. SECURITY CLASS. (of this report)
UNCLASSIFIED

15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)
This document has been approved for Public Release and Sale. Its distribution is unlimited.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)
Plasmids, Transposons, Genetic Recombination, Regulation, Genetic Control, Gene Intergration

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)
Intermolecular exchange of a DNA segment, that is, genetic exchange or crossing over between homologous parental chromosomes resulting in the formation of a hybrid molecule, has long been recognized. This marvelous process is important in providing the breadth of phenotypic diversity seen within a single plant or animal species. Classical genetics and recent studies in molecular genetics have revealed a variety of genetic exchange systems in bacteria. These recombination systems can be divided into two broad categories: (a) generalized, and (b) specialized. In short, general recombination systems mediate genetic—

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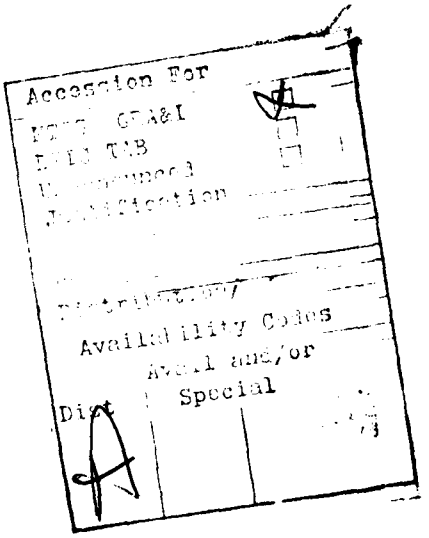
interchange at random points between largely homologous deoxyribonucleotide segments, whereas specialized recombination processes act in the absence of general recombination or apparent sequence homology between the interacting DNA regions. Specialized recombination systems are responsible for promoting the integration, deletion, transposition, or inversion of discrete DNA segments and can also influence the expression of nearby genes.

Bacterial evolution was thought until recently to occur by a slow process involving small chromosomal alterations (i.e., mutations), environmental selection for the desirable mutations, and the accumulation of beneficial mutations through intercellular genetic exchange and generalized recombination. Mutational events which involve the addition, deletion, or substitution of only one or a few nucleotides can be called micro-evolutionary. Since the 1960's, however, a catalog of macro-evolutionary events has been amassed. These involve the reshuffling, often at relatively high frequencies, of large chromosomal DNA segments, and include inversion, insertion, duplication, deletion, or transposition events, as well as the chromosomal integration of circular DNA. Micro-evolutionary alterations probably occur mainly during DNA replication or repair or both, whereas macro-evolutionary events are mediated largely by a variety of specialized recombination systems. Bacterial evolution probably proceeds by the accumulation of both micro- and macro-evolutionary changes. However, it should be stressed that unlike micro-evolutionary alterations, macro-evolutionary rearrangements often affect the expression of many genes and occasionally do so in an irreversible manner (e.g., sequence deletion). Hence, specialized recombination systems, which appear to contribute significantly to overall bacterial evolution, offer novel mechanisms with which bacteria can cope with the forces of natural selection. This review is intended as a brief description of several of the more intensively studied specialized recombination systems, and relates how these processes affect both gene expression and evolution in bacteria.



Specialized Genetic Recombination Systems in Bacteria: Their Involvement in Gene Expression and Evolution

D. J. Kopecko

A. Introduction

A variety of phenomenal DNA units, aided by specialized recombination processes, are responsible for a major proportion of the "spontaneous" chromosomal alterations observed in bacteria. These structurally and genetically distinct DNA segments (i.e., bacterial viruses, insertion sequence elements, and transposons) can be inserted within many chromosomal loci. In addition to causing insertion mutations and encoding new genetic potential, these discrete units act as supernumerary genetic regulatory switches capable of enhancing or eliminating the expression of nearby genes. Furthermore, recombination promoted by these DNA elements can result in various chromosomal rearrangements affecting large or small chromosomal DNA regions and involving the joining of unrelated DNA regions that lack apparent nucleotide sequence homology. Though this review is aimed at describing the intensive study of recombination mediated by viruses and transposable elements in bacteria, there is considerable evidence to suggest that transposable elements are also significantly involved in genetic reorganization and regulation in higher organisms (McClintock 1957; Bukhari et al. 1977; Kleckner 1977; Starlinger 1977; See Sect. F). Intermolecular exchange of a DNA segment(s) (i.e., genetic recombination or crossing over) between homologous parental chromosomes, resulting in the formation of a hybrid molecule, has been recognized in eukaryotic systems since the early days of classical genetics (Hayes 1968). This marvelous process is important in providing us with the breadth of phenotypic diversity that one sees within a single plant or animal species. More fundamentally, recombination promotes new genetic combinations upon which the forces of natural selection can act, eventually leading to the evolution of an organism more suited to the environment. Unfortunately, the multichromosomal organization of eukaryotic hereditary information as well as the absence of experimental methods to manipulate this material have precluded, for the most part, molecular analyses of either mutations or various recombinational events in higher organisms. The recent "genetic engineering" techniques lay the foundation for fine structure study of eukaryotic chromosomes, but little has yet been accomplished along these lines. However, the eugonic bacteria and their viruses, each containing one relatively simple chromosome, seem to have been specially constructed for the molecular geneticist. Bacteria are relatively simple, undifferentiated organisms that reproduce by asexual fission, a process characterized by the doubling of the cellular

contents followed by the equipartitioning of the replicated hereditary information at cell division. Thus, each daughter cell is essentially a genetic replica of the parent. Despite the absence of sexual reproduction in bacteria, intercellular exchange of genetic information can occur readily, not only between different bacterial species, but also intergenerically (for review see Hayes 1968; Lewin 1977; Kopecko et al. 1979). Beyond the apparent evolutionary significance of this intercellular genetic exchange, bacterial genetic transfer processes (e.g., conjugation) have been successfully manipulated to obtain our present understanding of the genetic and molecular organization and expression of hereditary material.

Bacterial evolution, until recently, was thought by many to occur by a very slow process encompassing the induction of a small alteration in a chromosomal DNA sequence (i.e., a mutation), environmental selection for the desirable mutations, and the accumulation of beneficial mutations through intercellular genetic exchange and general recombination (see Fig. 1). This concept was fostered by the results of early genetic studies in which bacteria were found to mutate relatively infrequently (i.e., one mutant per 10^6-10^8 cells for any given trait) and each "spontaneous" mutation appeared to represent an alteration in only one

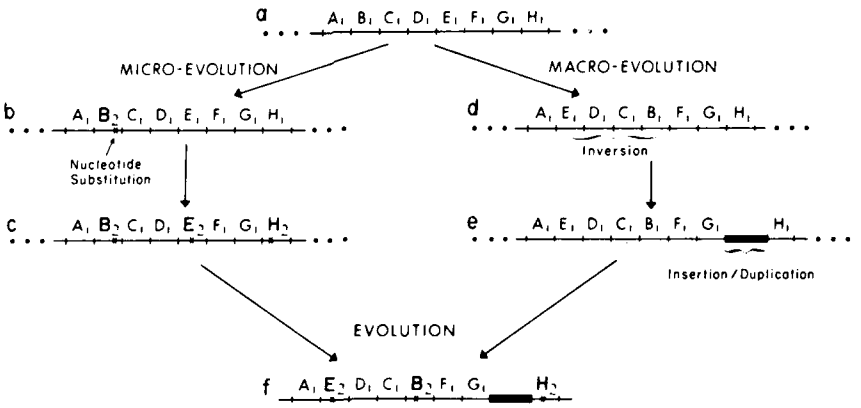


Fig. 1. Schematic representation of evolution. The horizontal lines represent a portion of the bacterial chromosome and arbitrary genes are labeled A, B, ... and H. The subscript, 1 or 2, after each gene designates an allelic form of that gene. The chromosomal segment shown in (a) can undergo small single nucleotide base changes or micro-evolutionary events, such as that shown in (b) and (c). The mutated genes are indicated by a subscript 2 and pronounced lettering, and the mutations are located by an *X*. In addition, large chromosomal or macro-evolutionary rearrangements such as the inversion of a DNA segment (d), the deletion of a DNA segment (not shown) or the insertion of a DNA sequence (e) can occur. Overall bacterial evolution appears to result from the accumulation, via genetic recombination, of both micro- and macro-evolutionary chromosomal alterations, as shown in (f)

or a few adjacent mucleotide base pairs of DNA. It seems appropriate to refer to these mutational events, which involve the addition, deletion or substitution of only one or a few nucleotides, as *micro-evolutionary* (Dobzhansky 1955, see p. 165; Cohen et al. 1978). Within the past 10-15 years, however, it has become apparent that large chromosomal rearrangements (e.g., duplications, deletions, inversions, and transpositions of multinucleotide DNA segments) occur in bacteria, oftentimes at relatively high frequencies. These events which result in the rearrangement, gain, and/or loss or large DNA segments can accordingly be described as *micro-evolutionary* (Dobzhansky 1955, see p. 166; Cohen et al. 1978) and certainly must account for a major proportion of bacterial evolution. It appears that bacterial evolution results from the temporal accumulation of both micro- and macro-evolutionary DNA alterations (see Fig. 1).

Genetic recombination in bacteria, described in more detail below, can be divided into two broad categories: (1) general and (2) specialized. In short, *general* recombination mechanisms mediate genetic interchange at random points between largely homologous deoxyribonucleotide segments. Following the intercellular transfer of mutated DNA segments, both micro- and macro-evolutionary changes can be stably incorporated into the recipient chromosome via general recombination (Fig. 1f). However, whereas micro-evolutionary mutational events are thought to be caused in vivo by errors in DNA metabolism, sometimes induced by intermediary metabolites or radiation exposure, macro-evolutionary DNA alterations appear to be generated via a variety of special recombinational processes. Moreover, these macro-evolutionary genetic exchange processes are functionally independent of known general recombination systems. In addition, the distinctive behavior (i.e., the lack of a requirement for extended homology between interacting DNA regions) of these macro-evolutionary processes allows them to be categorized as *specialized* recombination systems.

Research on macro-evolutionary chromosomal alterations has only recently shifted from the purely descriptive to the mechanistic level. However, the recent results of electron microscope and DNA sequencing studies of various macro-evolutionary events have suggested that several distinct specialized recombination systems are functionally related, a finding that appears yet to be appreciated by many. Thus, my primary intent in this review is to describe for the non-specialist the various types of bacterial specialized recombination systems and to relate these processes to the overall scope of bacterial heredity (i.e., mutation, gene regulation, genetic exchange, and evolution). Although this review is slanted toward a general introduction to this rapidly unfolding area of specialized genetic recombination, I hope that the coverage is in adequate depth and breadth to be of value also to the genetic specialist. For the novice, several recent cursory and detailed surveys of bacterial heredity and genetic nomenclature are recommended for reference (Demerec et al. 1966; Hayes 1968; Hershey 1971a; Falkow 1975; Novick et al. 1976; Watson 1976; Lewin 1977; Luria et al. 1978; Kopecko et al. 1979). Because of the broad scope of this review

only representative research reports and review articles have been referenced. In fact, there are several excellent detailed literature reviews of various specific aspects of specialized genetic recombination and these references have been included in the appropriate sections below.

This review is organized as follows. General and specialized recombination are defined in the following section. Then, I have briefly reviewed the currently perceived genetic and molecular bases of general recombination in order to ensure that the significance of specialized genetic interchange is fully appreciated. Afterwards, several specialized recombination systems are discussed in detail and, finally, the inherent evolutionary significance and practical experimental application of these processes is discussed.

B. Genetic Recombination: An Overview

I. General vs Specialized Recombination

"Genetic recombination" refers to a variety of DNA exchange of processes, in different organisms, that result in heritable altered linkage relationships of genes or parts of genes. For instance, reciprocal exchange of DNA segments between largely homologous regions of similar chromosomes, a recombinational capability of most organisms, can be represented by ...abCDe... X ... ABcdE... = ...ABCDE... + ...abcde..., where similar upper and lower case letters denote, respectively, dominant and recessive forms (alleles) of the same gene on separate chromosomes. In addition to this simple reciprocal exchange between homologous DNA regions, presently recognized recombination processes encompass such diverse events as the chromosomal integration of entire extrachromosomal genetic elements such as bacterial plasmids or viruses (e.g., the F plasmid and phage λ) as well as chromosomal gene duplication (e.g., ...ABABCD...), inversions (e.g., ...ABDCEF...), delections (e.g., ...ABDBCD...), and transpositions (e.g., ...ADEFBC...). Albeit varied, these events are all mediated by one of two classes of recombinational activities. *General* recombination mechanisms mediate random genetic exchanges between largely homologous segments on the same or on different genomes and require certain host general recombination functions. As a result of general recombination, virtually any DNA segment can be exchanged, but only between homolgous DNA regions. In contrast, *specialized* recombinational activities act independently of general recombination functions and do not require large regions of homology between interacting DNA segments. Certain *specialized* recombination processes catalyze the integration and/or excision at a limited number of chromosomal sites of physically defined genetic units (e.g., λ phage integration into the *E. coli* genome), or, in other cases, insertion or deletion of discrete DNA segments at seemingly random chromosomal loci (e.g., Mu phage or transposon insertion into the *E. coli* genome). In addition to the currently described specialized

recombination systems that mediate the genetic exchange of recognizable, defined DNA units (e.g., λ or Mu bacteriophage; insertion sequence elements), present evidence would lead one to believe that these or similar specialized recombination systems promote the occasionally observed exchanges, at variable loci, between bacterial DNA segments that lack apparent nucleotide sequence homology (e.g., "spontaneous" chromosomal deletion or transposition events; and specialized transducing phage formation). General recombination and the various specialized recombination systems appear to be mediated by separate overall processes, but may share common components of DNA metabolism, such as winding/unwinding enzymes, ligase, polymerases, various nucleases, and DNA binding proteins.

II. Genetic Aspects of General Recombination

Particularly noteworthy prerequisites to our current conceptualizations of genetic recombination in bacteria were the discoveries of DNA structure and the informational organization of DNA, as well as the various mechanisms whereby bacteria can exchange their hereditary material intercellularly (Hayes 1968; Watson 1976). Upon the introduction into recipient bacterial cells of large segments of a similar (donor) bacterial chromosome via conjugation, or of smaller bacterial DNA fragments via transformation or transduction, normally haploid recipient cells become partially diploid (i.e., merodiploid) for the DNA segment transferred. Merodiploid cells are usually genetically unstable and characteristically lose the newly inherited trait. One can observe this loss if the donor DNA segment, ...ABDdef..., is phenotypically distinguishable from the analogous recipient chromosomal region, ...abcDEF... . However, with a probability of ≈0.5, these merodiploid cells can undergo a genetic recombination event(s) in which a random segment of the newly inherited material is exchanged for an analogous portion of the recipient chromosome. If, for instance, the dominant ABC alleles were inserted in place of the recessive abc alleles of the recipient, the resulting recombinant bacterial genome would be dominant for the region ...ABCDEF..., and all progeny bacteria would inherit this recombinant genotype (Hayes 1968). This random recombinational exchange of DNA segments between largely homologous interacting DNA regions is thought to occur universally among bacteria. However, most intensive genetic analyses of this general recombination phenomenon have been conducted in the well characterized *Escherichia coli* K-12 system.

The insightful genetic studies of A.J. Clark and others (see Clark 1973; Lewin 1977; Mahajan and Datta 1979) have established the specific involvement of several recombination [illegible] genes in this process. Through genetic complementation analyses of various mutants in recombination deficient (Rec⁻) [illegible], Clark (1971) has deduced the normal existence of two general recombination pathways, both of which require the 40,000 mol. wt. [illegible] product. One pathway that utilizes, in addition to the [illegible] protein product, the *recB*, *C* exonuclease, normally accounts for [illegible] general recombination. In cells deficient in the [illegible],

nuclease, a second pathway involving the uncharacterized *recF* gene mediates recombination at about 1% of wild-type levels. There is now considerable evidence to suggest that the *recB, C* recombinational pathway is involved primarily in double-strand genetic exchanges, while the *recF* pathway mediates mostly single-strand DNA exchanges (Mahajan and Datta 1979). In addition, separate studies have revealed similar phage-specified general recombination systems in λ, T4, T7 and P22 (Lewin 1977).

III. Molecular Mechanisms of Homologous Recombination Processes

Little is factually known about the molecular events involved in recombination. However, there is considerable evidence to indicate that general recombination encompasses physical breakage of parental molecules and reunion of exchanged DNA segments (see Lewin 1977). Stahl and co-workers have recently provided evidence for the existence of *recB, C*-dependent, randomly located (about every 5000 base pairs) recombination sequences, called Chi, on the *E. coli* chromosome (Stahl et al. 1975; Malone et al. 1978; Chattoraj et al. 1979). Although not yet proven, physical pairing of interacting DNA regions (i.e., recombinational synapse) may be catalyzed by the *recA* protein (Shibata et al. 1979), while the Chi sequences may be involved as enzyme recognition sites in the final resolution of the hybrid structure. Molecular models for general recombination are necessarily speculative, but several have been included here to generate a general concept of events likely to be involved in recombination and for later comparison and contrast to specialized recombination mechanisms. Figure 2 diagrammatically depicts events likely to be involved in the integration of a single linear DNA strand, acquired by conjugation or transformation, into the bacterial chromosome. Experimental results suggest that entering donor single-stranded DNA quickly pairs with a homologous region on the chromosome. The donor single-strand is exchanged with the recipient molecule at a gap either created by general recombination enzymes or remaining from DNA replication or repair activities. Following nuclease trimming of the non-exchanged ends of the donor strand, covalent closure of the newly constructed recombinant might involve repair synthesis or simply ligation. The exchange of a single DNA strand between two double-stranded (duplex) DNA molecules, as shown in Figure 1E, could occur by a minor variation of the scheme shown for single-strand integration. If the complementary strands in the hybrid region of the recombinant molecule differ, DNA repair mechanisms might remove any mispaired bases. Alternatively, replication of the hybrid molecule would generate daughter chromosomes that differ in the region of the original recombinational event. Following single-strand DNA transfer to a recipient cell via conjugation, segments as large as 500,000 nucleotide bases in length have been detected by genetic means to be incorporated by general recombination. Although hybrid molecules are formed in the absence of DNA synthesis, the final covalent linkage of exchanged strands in the hybrid molecule appears to require cell growth, but the specific requirements for DNA, RNA, or protein synthesis are unknown (reviewed by Lewin 1977).

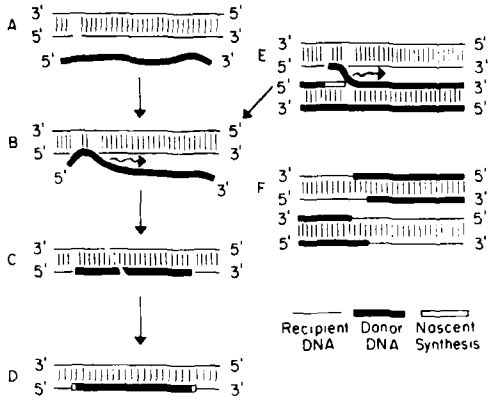


Fig. 2. Molecular models for general recombination. Interacting DNA strands are indicated by *horizontal lines*; hydrogen bonds between complementary nucleotides are shown as *short vertical lines*. Steps A-E describe events likely to occur during the integration of a single DNA strand into a recipient double-strand molecule. (A) Entering single-strand donor DNA quickly pairs with a homologous region of the chromosome. Genetic exchange is initiated on the recipient genome at a single-strand gap created by a recombination enzyme(s) or some other DNA metabolic activity. (B) Extension of the exchanged region (see *gap area*) following displacement and/or degradation of the corresponding recipient strand. (C) Termination of the genetic exchange may occur at a gap introduced by a specific recombination enzyme or by some other event. Unincorporated donor sequences are exonucleolytically removed. (D) The gaps on each side of the incorporated DNA segment are repaired by DNA polymerase and ligase. Any differences (base mispairings, nucleotide additions or deletions) between the strands of these recombinant DNA molecules are either corrected by DNA repair processes or expressed following replication. (E) The exchange of a single DNA strand between two double-stranded DNA molecules could occur in a manner similar to that described above, except that exchange between paired regions would require, at least, single phosphodiester bond cleavages in corresponding strands of both donor and recipient molecules. Incorporation of the exchanged DNA segment would occur as shown in steps B-D. Complementary donor strand synthesis could occur subsequent to or simultaneously with displacement of the donor single strand. Alternatively, (not shown) a single strand from each molecule could exchange with the opposite molecule giving rise to a reciprocal exchange of single DNA strands. (F) This diagram depicts the products expected from a single reciprocal, double-strand exchange between two linear DNA molecules. It should be noted that chromosomal integration of the F plasmid or phage lambda occurs by a specific, single, reciprocal, double-strand exchange between two circular molecules and results in a large composite circle. Two reciprocal exchanges would have to occur in order to exchange any DNA segment between two circular genomes. Reciprocity in genetic recombination is explained in the text.

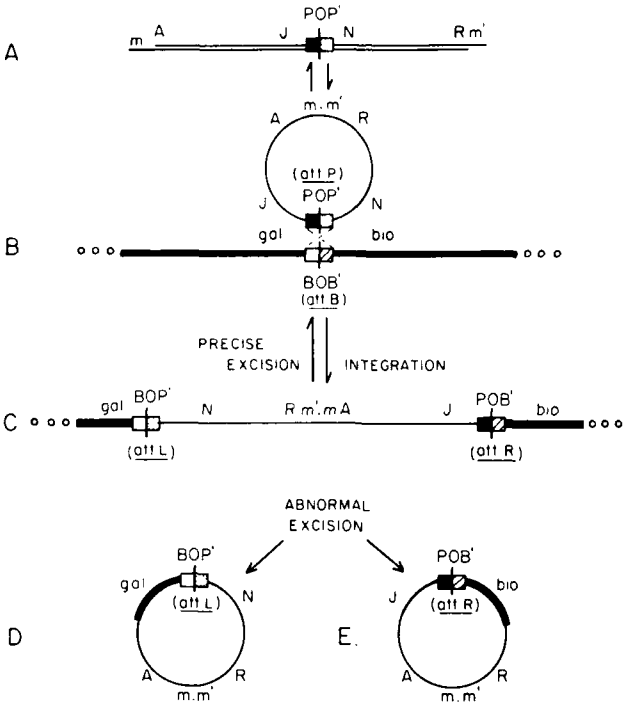


Fig. 3. Integration and excision of phage λ. This unscaled diagram depicts various molecular states of the temperate bacteriophage λ. The phage chromosome, normally ≈45 kilobase pairs in length, is represented by a [illegible] while the [illegible] correspond to segments of the bacterial chromosome. The [illegible] descriptively labeled POP' and BOB' represent the analogous attachment sites on the phage (att P) and bacterial (att B) chromosomes, respectively. The common core sequence, O, is depicted by a [illegible] in the middle of each att site whereas the unique flanking sequences P, P', B and B' are represented by the [illegible]. The DNA sequences marked with A, J, N, and R are phage structural genes while m-m' is the site on λ which is endonucleolytically cleaved giving rise to single-stranded, 12 nucleotide long complementary (cohesive) 5' ends, m and m'. The only bacterial genes shown are those for utilization of galactose (gal) and the synthesis of biotin (bio), which are situated nearby to the primary att B site in E. coli. (A) The linear λ genome. This is the form in which λ DNA is packaged in the virion. During packaging, phage monomers are excised from tandem oligomeric replicative forms by specific endonucleolytic cleavage at symmetrically identical sites that are separated by 12 nucleotides in opposite strands at the cos (m-m') locus. Both strands of λ DNA are depicted in this section to emphasize the cohesive termini. In steps B through E, both λ and bacterial DNA are depicted by single lines of different width. (B) Phage circularization and lysogenization. Once injected intracellularly, linear λ circularizes by ligation of the complementary termini, m and m'. Phage integration occurs after pairing of the analogous attachment regions on both λ (POP') and the host (BOB') genomes, by a specific recombinational crossover mediated by the λ-coded integration (int) protein. (C) λ prophage. The normal linear arrangement

Exchange of a double-stranded segment between two duplex DNA molecules, although more complex, might follow a course of events similar to that described above. However, the requirements of the exchange would depend upon the physical state (i.e., circular or linear) of the interacting molecules. Also, the exchange could be *reciprocal*, an event in which all DNA ends created by recombinational cleavage are rejoined to new sequences, or *non-reciprocal*, an event in which new DNA ends are generated by the recombinational event. The end product of a single reciprocal double-strand exchange is shown in Fig. 2F. Note that two reciprocal, double-strand crossover events are required to exchange a single contiguous DNA segment via generalized recombination between two circular molecules, which is the normal physicochemical structure of bacterial, plasmid, and many virus genomes. In contrast, one reciprocal, double-strand exchange between two circular molecules would produce one larger composite circular molecule, the product observed for the chromosomal integration of viruses or plasmid (see Fig. 3B, C). Additionally, the extent of homology between the F plasmid and the bacterial chromosome that results in *recA*-dependent integration of F is now known to be approximately 1000 nucleotide base pairs (Davidson et al. 1974). Thus, only a relatively small amount of sequence homology between interacting molecules is needed for homologous recombination.

General recombination mechanisms, then, mediate the physical exchange of single or double-stranded DNA at random points between paired and largely homologous DNA segments. In *E. coli*, general recombination is specifically dependent upon the *recA* gene product, but other DNA metabolic activities are also involved (Radding 1973; Clark 1974; Lewin 1977). The recent cloning of the *recA* gene and purification of its protein product (McEntee and Epstein 1977) in conjunction with the findings of Stahl and co-workers (Malone et al. 1978) of the Chi sequences and the recent direct visualization by electron microscopy of synapsed DNA regions (Potter and Dressler 1979) would suggest that some of the events involved in generalized genetic exchange may soon be deciphered. Hopefully, this brief overview of general recombination will better enable the reader to comprehend the catalog of "aberrant" or specialized recombination events described in the following sections.

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of the prophage genome within the host chromosome is shown. This integration process is reversible, and upon induction, precise excision of the λ genome is effected by both *int* and the excision (*xis*) proteins, resulting in the production of normal λ phage (see step B). Occasionally, however, prophage excision is inexact and creates a defective specialized transducing phage (see D and E). (D) Circular λ *dgal*. During excision of the λ prophage, the recombinational crossover took place between a site within λ and a site in the *gal* region of the chromosome giving rise to a defective phage particle which has lost a set of phage genes, but gained a corresponding length of bacterial DNA, in this case the *gal* genes. (E) A similar abnormal excision involving the opposite end of the λ prophage can create λ *dbio* transducing phages.

C. Specialized Recombination Systems in Bacteria

I. Introduction

Atypical recombination events in bacteria were first characterized in depth during the study of the temperate bacterial viruses. For example, in contrast to the randomness of general (or legitimate) recombination events, bacteriophage λ was observed to integrate itself physically as a linear DNA addition into a specific site on the *E. coli* K-12 chromosome. Although upon induction, prophage λ excision from the integrated state was most often precise, occasionally an imprecise event would take place in which part of the λ sequences would excise along with some adjacent bacterial DNA giving rise to a specialized transducing phage (Weisberg et al. 1977; see Fig. 3). Furthermore, entirely separate studies conducted during the 1960's revealed that reshuffling of large segments of the *E. coli* chromosome through duplication, deletion, inversion, or transposition could occur (see reviews by Starlinger and Saedler 1976; Starlinger 1977). Although seemingly different, all of the above events required little or no homology between interacting DNA regions and could occur in bacteria deficient in general recombination ability (e.g., *recA*-deficient *E. coli* K-12). This *recA*-independent, physical joining of two apparently non-homologous DNA segments, once thought to be a rare, aberrant event, has previously been termed "illegitimate" recombination (Franklin 1971). These processes result in the formation of a novel joint as two grossly unrelated DNA regions are fused (Hershey 1971a).

More recently, a series of discrete DNA segments, called transposable elements, have been identified which can transpose independently of host *recA* function, intra- or inter-molecularly. These elements have been found to promote many of the macro-evolutionary events described above, as well as to affect gene expression by causing insertion mutations or by carrying DNA sequences that act as genetic transcriptional promoter and/or termination signals (Starlinger and Saedler 1976).

"Illegitimate" recombination was an amorphous discipline with limited examples at the time of Franklin's comprehensive review (1971). During the last decade, due specifically to the development of electron microscope heteroduplexing and denaturation mapping procedures (Westmoreland et al. 1969; Inman and Schnos 1970; Davis et al. 1971), the discovery of many site-specific DNA endonucleases (reviewed by Roberts 1976), and advances in DNA sequencing techniques (Maxam and Gilbert 1977), we have uncovered and defined a series of what appear to be different specialized recombination systems that are responsible for some of the events heretofore termed "illegitimate". This section will entail general descriptions of representative examples of various specialized recombination systems. However, some macro-evolutionary rearrangements will be described for which no known specialized genetic exchange mechanism has yet been implicated. These events which occur in the absence of general recombination and extended DNA homology will be referred to as "aberrant" or "illegitimate".

II. Bacteriophage λ

1. Site-specific Recombination System

The temperate bacteriophage λ is normally packaged in the virion as a linear, double-stranded DNA molecule, as diagrammed in Fig. 3A, which has been enzymatically cleaved by a specific endonuclease that creates 12 nucleotide-long complementary (cohesive) 5' ends, *w* and *w'*. Following bacteriophage infection of a bacterial cell, the injected phage DNA molecule stably circularizes by ligation of its reannealed cohesive ends. Either lytic replication can ensue or, by definition, a temperate bacterial virus can exist intracellularly in a quiescent (i.e., lysogenic) state from which it can later be induced to undergo lytic/vegetative growth. During lysogenization the functions needed for λ lytic growth are repressed. In addition, a site-specific reciprocal recombination event occurs between a specific attachment/recognition site, called *att*P, on the circular phage DNA and an analogous receptor site, *att*B, on the bacterial genome, resulting in the ordered linear insertion of λ into the *E. coli* chromosome (see Fig. 3B,C). This event requires a phage-encoded integration (*int*) protein, which has a subunit molecular weight of approximately 40,000 daltons, that binds to specific sites within *att*P and is known to have DNA nicking-sealing activity (Nash 1977; Kikuchi and Nash 1978; Nash, personal communication). The λ prophage, which is now replicated as an integral part of the bacterial genome, is bounded by hybrid attachment/recognition sites which have been designated *att*L (left) and *att*R (right; see Fig. 3C). No extended regions of homology could be detected among *att*P, *att*B, *att*L and *att*R by electron microscope heteroduplex procedures (Hradecna and Szybalski 1969; Davis and Parkinson 1971), but recent direct DNA sequence analysis has shown that each of these four *att* sites has the following 15 deoxyribonucleotide base common core: 5' -GCTTTTTTATACTAA- 3' (Landy and Ross 1977). Despite the presence of the common core region, however, the sequences on either side of the core in *att*B or *att*P are different from one another. Because λ integration is a reversible process, this common core region must be the physical locus at which integrative recombinational DNA breakage, exchange, and reunion occur. As a result of the different core-adjacent sequences, each *att* site is genetically distinct, i.e., each displays a unique set of affinities for the other *att* sites during integrative/excisive recombination events (Parkinson 1971; Nash 1977). The overall organization of the *att* sites and exact size of the core-adjacent sequences which affect site-specific recombination are not yet known. Although λ normally integrates into a primary *att*B locus located at 17 min on the *E. coli* genetic map (Bachmann et al. 1976), in bacterial hosts containing a deletion of this primary bacterial *att* site λ will integrate less efficiently into a variety of secondary bacterial *att* sites. These secondary sites appear, in all respects, to be natural variants of *att*B (Shimada et al. 1972) and when λ is integrated at a secondary site that lies within a detectable gene, the λ prophage acts as a large insertion mutation within that gene.

At induction λ prophage repressor is inactivated, possibly through proteolytic cleavage by the host *recA* protein (Roberts et al. 1979), allowing λ gene expression which is normally followed by excisive recombination between the hybrid *att* sites, an event that appears essentially to be the reverse of integration (see Fig. 3B,C). Excision requires the action of two phage-encoded products, the *int* protein and an excision (*xis*) gene product (encoded by < 250 base pairs; Nash 1977), and results in the generation of circular bacterial and λ chromosomes. Neither phage nor host general recombination abilities or phage genes other than *int* and *xis* appear to be involved in this site-specific specialized recombination system. Furthermore, λ integration and excision can occur in the absence of DNA, RNA or protein synthesis. Thus, in contrast to our current knowledge of general recombination mechanisms, these integration/excision events must not involve exonucleolytic trimming of exchanged DNA, the random creation of single-strand gaps, or gap-filling by nascent DNA synthesis (Kikuchi and Nash 1978). Recently, mutant λ phage that simultaneously carry *att*P and *att*B, or *att*L and *att*R, have been constructed. These mutant viruses participate in both in vivo and in vitro inter- and intra-molecular site-specific recombination reactions as illustrated in Fig. 4 (Engler and Inman 1977; Nash 1977; Nash et al. 1978). Data obtained with

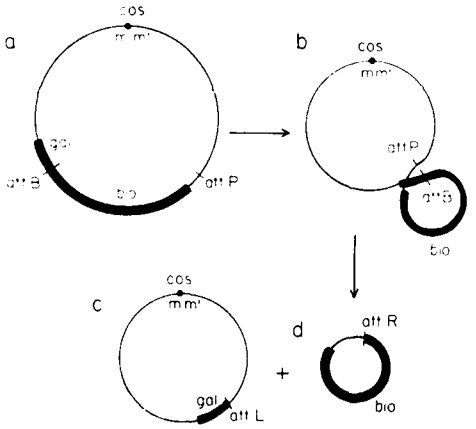

Fig. 4. λ *att*B-*att*P, substrate for integrative recombination in vitro. (a) The unusual transducing phage carrying both *att*P and *att*B was reported by Nash 1974. Lambda sequences (*att*P; *cos*) are represented by a thin line and the bacterial sequences (*gal*, *bio*, *att*B) carried by the phage are indicated by a thicker line. (b) Intramolecular recombination between *att*P and *att*B generates two smaller circular molecules, c and d. (c) Viable λ transducing phage carrying the bacterial *gal* genes plus the hybrid *att*L site. (d) A small non-self-replicating circle carrying the *bio* gene segment plus the hybrid *att*R site. Other λ transducing phage carrying one or more of the *att* sites (*att*P, *att*B, *att*R and *att*L) have since been isolated and are extremely useful in biochemical analyses of site-specific integration and excision (for details see Nash et al. 1978)

these and other mutant phage indicate that λ, through the action of *E. coli* ligase and gyrase, must exist as a covalently-sealed, negatively supertwisted circle in order for integration to occur (Mizuuchi et al. 1978).

Beyond the requirement for host ligase and gyrase, several *E. coli* genes have been identified by mutations that affect the λ site-specific integrative recombination process. The host integration mutations *(him)* have been mapped at 38 min *(himA)*, 84 min *(himB)*, and at 20 min *(hip* or host integration protein) on the *E. coli* genetic map (Miller and Friedman 1977; Nash et al. 1978). Interestingly, these mutations also affect other specialized recombination processes and these genes may comprise a series of proteins common to DNA metabolism (i.e., repair, replication, and recombination). Although the mechanisms of λ integration/excision events have not been physically defined, Landy and Ross (1977) have pointed out several features of the *att* sites (e.g., direct and inverted repeat DNA sequences, as well as adenine plus thymine rich regions) that might influence their specific recombinational behavior (see later section on mechanism of transposition).

Thus, bacteriophage λ encodes a specialized recombination mechanism that enables λ, as a discrete genetic unit, to integrate site-specifically into and excise from one or a limited number of sites on the *E. coli* genome. In addition, λ codes for a random genetic exchange system, specified by the λ *red* genes, that is analogous to the bacterial *rec* system and is not required for λ phage integration or excision. Though not mentioned above, complex genetic regulation of λ gene expression controls the fate of the infecting phage (i.e., lytic or lysogenic state). Several recent excellent reviews of these regulatory controls as well as of λ integration/excision are available (Gottesman 1974; Campbell 1976; Schwesinger 1977; Nash 1977; Weisberg and Adhya 1977; Weisberg et al. 1977; Nash et al. 1978).

In addition to performing precise integration/excision functions, the λ site-specific recombination system is able to promote recombination between two autonomous phage chromosomes (Table 1). Such events are *int*-dependent, occur only at the *att* sites (presumably via integrative recombination) and can take place in the absence of λ Red or host Rec general recombination (Weil and Signer 1968; Echols et al. 1968). For example, in a cell doubly infected with two λ derivatives that are genetically marked by mutations on opposite sides of *attP* in each phage type, one can detect reciprocal recombinant phage which carry approximately half of each parent phage sequence, with the recombination event occuring at *attP* on each molecule, presumably between linear phage molecules. Furthermore, this specialized recombination system also appears to be involved in the occasional formation of certain site-specific phage deletion mutants. Formation of these phage deletions, in which one deletion end point always occurs at *attP*, has been shown to require the λ *int* protein. Davis and Parkinson (1971) proposed two mechanisms to explain this infrequent deletion formation: (1) exonucleolytic digestion from a nicked *attP* site followed by joining the ends of the

Table 1. Specialized recombination events involving λ phage

Type of event	Functions and sites		Physical state of phage		Final State of recipient chromosome	Frequency	References
	Required	Not required	Initial	Final			
Site-specific phage integration	*int*, *att*P *att*B	*xis*, *rec* *rec*A	Autonomous phage	Prophage inserted in one orientation at *att*B	Linear, ordered insertion of the phage at *att*B		Hershey (1971a) Gottesman (1974) Campbell (1976) Nash (1977) Nash et al. (1978)
Precise prophage excision	*int*, *xis*, *att*R, *att*L –	*rec* *rec*A	Prophage	Autonomous phage	Original host sequences restored upon prophage excision	All prophage can be excised after induction	Hershey (1971a) Gottesman (1974) Campbell (1976) Nash (1977)
Site-specific exchange between λ phages	*int*, *att*P and/or other *att* sites –	*rec* *rec*A	Two autonomous linear phages?	Two recombinant phages, each carrying reciprocal halves of the parental molecules, with the exchange beginning at *att*P	–		Echols et al. (1968) Weil and Singer (1968) Lewin (1977)
Site-specific phage deletions	*int*, *att*P –	*rec*, *xis*? *rec*A	Autonomous phage	Autonomous phage containing a deletion with one end point at *att*P: the other end point can be fixed (e.g., λ*b*2) or random	–	5×10^{-6} events/cell	Davis and Parkinson (1971) Parkinson and Huskey (1971) Weisberg and Adhya (1977)

Imprecise prophage excision or transducing phage formation	-	*int*, *xis* *red*, *attR* *attL*	Prophage	Specialized transducing phage carrying bacterial sequences from one or both sides of *attB*	Chromosomal deletion; part or all of prophage is removed along with adjacent bacterial sequences	10^{-5}-10^{-3} events/ cell, after induction	Hershey (1971a) Weisberg and Adhya (1977)
	-	-					
Aberrant deletion formation	?	*red*, *int*, *xis*, *att* sites	Prophage	Prophage containing a non-specific deletion encompassing λ sequences involved with cell lysis & also some adjacent bacterial sequences	Chromosomal deletion; part of the prophage and some adjacent host sequences are deleted	10^{-7}/cell after a single phage growth cycle	Weisberg and Adhya (1977)
	-	*red*					
Internal λ deletion or λ formation	*int*, *xis*	*red*	Replicating autonomous phage; e.g., λ*dv*	Small defective phage containing large internal deletions of normal λ sequences; e.g., λ*dv*	-		Matsubara and Kaiser (1968) Berg (1974) Chow et al. (1974)
	-	*red*					
DNA sequence duplication within λ phage	-	*red*	Autonomous phage containing a deletion	Autonomous phage containing a tandem direct duplication	-	10^{-4} events/ cell	Emmons et al. (1975) Weisberg and Adhya (1977)
	-	*red*					

Occurrence of the above events in the absence of phage *red* or host *rec* general recombination systems is the unifying characteristic of these genetic exchanges. Those functions/sites that are known to be required or that are not necessary for the above events include: phage integration (*int*), excision (*xis*), and *red* functions and the attachment sites (*attP*, *attR* and *attL*); bacterial *rec* function and the attachment site (*attB*). The pre- and post-recombinational states of the phage and the effect, if any, on the bacterial chromosome is given for each type of event. The recombinational systems involved in the latter four events are unknown

digested strand as shown in Fig. 5a-c, and (2) an *int*-promoted unequal crossing-over event in which a region beginning at *att*P on one molecule was exchanged at a non-homologous secondary site on another phage molecule (Fig. 5d-f). The involvement, if any, of *xis* in λ site-specific deletion formation is unknown, but one can envision *int* and *xis*-dependent deletions which might occur by intramolecular deletion of a sequence between *att*P and certain specific secondary sites in λ. Such an intramolecular deletion is exemplified by the exchange between *att*P and *att*B on the special λ *att*P-*att*B phage, as illustrated in Fig. 4. Current evidence seems to favor this latter mechanism: Shimada et al. (1972) have observed *int*-promoted inefficient recombination between *att*P and secondary *att* sites in bacteria lacking the primary *att*B locus; several apparently identical *int*-dependent deletions of λ, call *b*2, have been isolated independently (Weisberg and Adhya 1977); and, finally, *int* and *xis*-dependent recombination events have been reported between each *att* site and any of the other sites (i.e., *att*B, *att*P, *att*L or *att*R), though the recombination frequencies varied widely (Parkinson 1971; Guarneros and Echols 1973; Lewin 1977; Nash 1977).

5. Transducing Phage Formation and Other Illegitimate Recombination

Imprecise or aberrant excision of a λ prophage occurs occasionally by a process(es) that takes place independently of *int* or *xis* functions, the prophage *att* sites, and the λ *red* or host *recA* genes (Weisberg and Adhya 1977). This process(es) yields specialized transducing viruses, at a frequency of ~1 per 10^6 normal

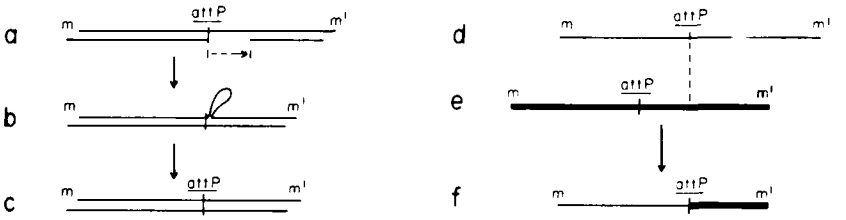

Fig. 5. Proposed mechanisms for *int*-dependent site-specific deletion formation in λ. Linear phage chromosomes are represented by *single lines* (d, e, f) or *double* (a, b, c) *horizontal lines*. The cohesive ends, m and m', as well as *att*P are shown on each molecule. As proposed by Davis and Parkinson (1971), *int*-dependent deletion formation can occur in two ways. Shown in steps a-c, exonucleolytic digestion - depicted by *dashed line* in (a) - from a specific single strand cleavage in *att*P is followed by joining of the ends of the digested strand (b). Revomal of the unpaired sequences, shown as a loop in (b), would generate a λ deletion mutant, as shown in c. Alternatively, unequal crossing-over between two λ phages (d, e) in which a region beginning at *att*P on molecule was exchanged (see *dashed lines*, d and e) at a non-homologous secondary site on another phage, resulting in a shorter recombinant molecule (f)

phage released following induction, whose genomes are composed of part of the original phage sequences and some of the adjacent bacterial genes (see Fig. 3D,E). Specialized transducing phage can be serially propagated, but sometimes only in the presence of coinfecting helper phage to supply missing essential phage functions. Each originally isolated transducing particle appears to be distinct from other transducing particles with respect to the extent of both phage genes remaining and bacterial genes substituted. This finding indicates that the recombinational crossover occurs at random points between the interacting phage and bacterial sequences. However, weak evidence exists to suggest that there are preferred sites in the bacterial DNA sequences adjacent to the prophage at which abnormal excision/transducing phage formation occurs (Weisberg and Adhya 1977). The low frequency of specialized transducing phage formation is probably a composite of (1) an inefficient recombination event(s), (2) a requirement that the transducing phage contain the cohesive ends, [illegible] and [illegible]', in order to be packaged, (3) the fact that fusion must occur between the phage and bacterial sequences at the abnormal site of recombinational crossover to generate a circle (or else scission at [illegible] during phage packaging would generate two fragments that could not be serially propagated), and (4) the necessity for the excised product to be between 0.73 and 1.09 [illegible] length in order to be packaged (Weisberg and Adhya 1977). No phage or bacterial genes have yet been identified that affect this abnormal excision process. It has been suggested that specialized transducing phages are formed at or after lysogenic induction, but the molecular bases for these apparently illegitimate events are currently unknown (Campbell 1963; Weisberg and Adhya 1977). However, since no extended region of homology exists between the interacting phage and bacterial DNA sequences, during aberrant excision, one could envision a phenotypically cryptic, chromosomally-determined specialized recombinational systems as being responsible for, at least, some transducing phage production (see later section on *recA*-independent bacterial recombination system). Therefore, transducing phage might be produced continuously at a low frequency by such a system, but detection would occur mainly following induction, when helper phage are expressed.

It is worth noting that a class of defective transducing λ phages, called λ*dg*L and λ*dg*R (*dg* = defective carrying one cohesive end), have been characterized (Little and Gottesman 1971). These phages, which cannot be serially propagated, carry bacterial sequences from the left or right side of *att*B plus half of λ and are generated after lysogenic induction, in part, by the site-specific cleavage of λ prophage at [illegible] (*bio*). Because the free left cohesive end of λ appears to be packaged first, the bacterial end of λ*dg*L is probably generated by the action of DNAses on the bacterial DNA protruding from the filled phage head. The process which results in the cleavage in the bacterial sequences of λ*dg*R is not adequately understood, but is thought to involve a nonspecific DNAse (for more detail see Weisberg and Adhya 1977). Thus, λ*dg* phages do not appear to be generated by recombinational mechanisms.

Lambda phage appears to be involved in several other types of illegitimate recombinational events, besides transducing phage formation (see Table 1). By examining bacterial survivors following induction of a heat-inducible λ lysogen, one can isolate bacterial deletion mutants which have lost part of the λ prophage (i.e., at least those λ genes involved in cell death) and some neighboring regions of the host chromosome. It is not known when (i.e., before or after phage induction) or how these deletions occur, but their formation is rare ($\sim 10^{-7}$/cell after one phage growth cycle), is not site-specific, and does not require λ *red*, *int*, *xis*, or *att* genes/sites (Weisberg and Adhya 1977). Again, a host-mediated, *recA*-independent recombination system may be responsible (see later section).

Vegetatively replicating λ*dv* or similar phage that express constitutive replication functions have been observed on occasion to undergo internal deletion of a large contiguous region creating λ*dv* (defective virulent) molecules (Matsubara and Kaiser 1968; Matsubara and Otsuji 1978). These non-integrative defective phage molecules, which can be formed in the absence of *recA* and *red* functions (Berg 1974), retain basically that part of λ which is normally essential for replication and responsible for immunity. Consequently, λ*dv* molecules lack most phage properties and exist intracellularly as multicopy circular plasmids comprising 50-250 copies/cell. Each separate λ*dv* isolate contains only from ~3 to 6 kilobase pairs of original λ information. However, λ*dv*'s often exist as dimers or higher multimers, consisting entirely of direct or inverted large tandem duplications (e.g., 3'...ABCABC...5' or 3'...ABCC'B'A'...5', respectively, where the primed letters represent the nucleotide sequence complements of the corresponding unprimed letters), sometimes interspersed by a unique DNA region (e.g., 3'...ABCDC'B'A'...5'). Based on electron microscope heteroduplex analyses, Chow et al. (1974) have hypothesized that λ*dv*'s arise from a partially replicated λ chromosome through recombinational events as depicted in Fig. 6.

In contrast to λ*dv* deletion mutants, other λ phage deletion mutants lose only short stretches of DNA. Because of the minimum DNA length requirements of λ packaging, it is not surprising that some λ deletion mutants often undergo partial genetic duplication which allows them to be packaged. Reiteration of some of the sequences in a λ phage deletion mutant does occur at a relatively high frequency ($\sim 2 \times 10^{-4}$ phage derivatives/cell after a single phage growth cycle; Emmons et al. 1975) compared to λ deletion formation. These duplication derivatives can easily be selected by their increased density in cesium chloride density gradients, by the increased concentration of a gene product, or by various genetic means (see Weisberg and Adhya 1977). Duplication occurs independetly of *recA* or *recB* gene products, possibly by an intramolecular recombination event between daughter arms of a partially replicated molecule or via intermolecular exchange (Weisberg and Adhya 1977). Many regions of the λ genome have been duplicated and the tandem direct repeat (3'...ABCBCDE...5') has been observed most often. Duplication mutants with direct repeats are genetically unstable and easily detected because the reiterated sequence can be lost by Rec- or Red-promoted recombi-

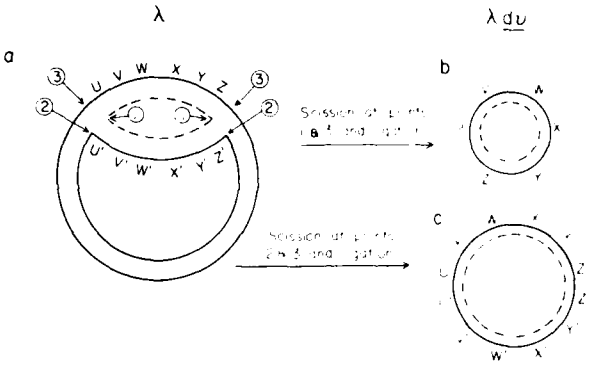

Fig. 6. Models for λdυ formation. Phage double-stranded DNA is represented by the two parallel circular lines. Dashed lines represent newly synthesized DNA. Arbitrary DNA sequences U through Z and the complementary DNA sequences, given in primed letters, are indicated. These illustrations are based on the models for λdυ formation proposed by Chow et al. (1974). By this proposal λdυ molecules are formed via scission immediately within or just outside of the termini of the replication fork and subsequent recA-independent recombination, resulting in the joining of parental to parental and progeny to progeny phage strands. (a) Bidirectionally replicating λ molecule. Breakage at the points marked by arrows 1 and 3 would generate a small linear fragment carrying the sequences U through Z. (b) λdυ formation could involve recA-independent recombination between the ends of this linear fragment and creation of a small circular molecule containing unique DNA sequences. (c) Scission of the replicating λ, shown in (a), at points 2 and 3 and subsequent joining of the original phage strands between sequences U and U', and Z and Z' would create a λdυ comprising a tandem inverted duplication. Note that single-strand interruptions may already exist at certain points in the replication fork due to the replication process, e.g., at points marked 1. Though not shown, a λdυ comprising a tandem-inverted dimer with interposed unique sequences at one or both ends of the repeated sequence could be generated by staggering the recombinational breakpoints at one or both ends of the replication fork (for details see Chow et al. 1974)

nation between the homologous regions (Bellett et al. 1971). Collectively, these data indicate that λ is involved in a series of seemingly aberrant recombinational events which occur independently of site-specific (i.e., int/xis), Red-mediated, or Rec-promoted recombination and that create specialized transducing viruses and internal phage sequence duplications, as well as deletions in autonomous or integrated λ and in adjacent bacterial sequences. As pointed out by Weisberg and Adhya (1977) in their recent review of illegitimate recombination events, more than one mechanism may be responsible for these aberrant exchanges involving λ. The various specialized and aberrant recombinational events in which λ phage are involved have been summarized in Table 1. The molecular maps of wild type λ and various derivatives as described above are included in the review of Szybalski and Szybalski (1979).

Albeit the bacteriophage λ integration/excision system is the best understood, other temperate bacterial viruses (e.g., Ø80, 434, 21, P2 and P22) are known to integrate via different specialized recombination systems at one or a few sites on the host chromosome and also to participate in many aberrant chromosomal rearrangements similar to those described above (Lewin 1977; Luria et al. 1978; Susskind and Botstein 1978). Therefore, when considering the causes of "illegitimate" recombinational events, one must be cognizant of the seemingly large assortment of phage-mediated specialized recombination systems and also of the types of genetic rearrangements that are promoted by these systems. Defective or non-inducible prophages or prophage remnants specifying specialized recombination systems may play a large role in bacterial macro-evolution. On the other hand, however, many genomic rearrangements observed in λ or other phages could be mediated by recombinational processes encoded by the bacterial host.

In contrast to the site-specific phage integration systems exemplified by λ, the unique temperate phage Mu can insert randomly into many chromosomal sites often causing detectable insertion (e.g., auxotrophic) mutations. Furthermore, this phage appears to be able to integrate into practically any phage, plasmid, or bacterial chromosome and can promote a variety of chromosomal reshufflings. Present knowledge of the Mu specialized recombination system, which represents the opposite end of the spectrum in recipient site specificity, is related in Sect. III.

III. The Mutator Bacteriophage, Mu

1. Genetic Definition

The novel bacterial virus, Mu, behaves as a temperate phage lysogenizing 5-10% of the cells which it infects or generating 50-100 infective virions per cell following the lytic cycle. Unlike most temperate viruses which integrate at one or a few specific host chromosomal sites, Mu can integrate at many, if not all, chromosomal loci. Consequently, about 2% of the cells lysogenized by Mu concomitantly acquire a new nutritional requirement or other recognizable mutation (Taylor 1963). This represents a frequency of mutation within a single gene of 50-100-fold higher than the spontaneous frequency observed in the absence of Mu. Both genetic and physical evidence indicates that these mutagenic events occur by the *recA*-independent, linear insertion of Mu into the affected gene (reviewed by Howe and Bade 1975). A series of studies on Mu integrated into several *E. coli* operons, mainly the lactose and tryptophan regions, has resulted in the following generalizations: (1) Mu-induced mutations are strongly polar, probably due to termination of transcription distal to the inserted phage (Jordan et al. 1968; Toussaint 1969; Daniell and Abelson 1973); (2) the mutations induced by Mu are extremely stable, with an apparent reversion frequency of $< 10^{-9}$-10^{-10} per viable cell (Taylor 1963; Jordan et al. 1968); (3) during lysogenization 10-15% of

the integrating Mu phage concomitantly cause deletions in bacterial sequences (see Howe and Bade 1975; discussed later in section on Mu-promoted deletions); (4) there do not appear to be preferred sites for Mu insertion within a gene, indicating that, if insertion is not absolutely random, the chromosomal attachment/recognition receptor sequence must only be two to three nucleotides long (discussed by Bukhari 1976; Couturier 1976); (5) non-transcribed genes or repressed operons are mutated by Mu approximately five times more often than actively transcribed DNA regions (see Howe and Bade 1975); (6) Mu phage and prophage genomes are collinear, i.e., all Mu prophage genomes have the same gene order as that found for the linear vegetative phage molecule (Howe and Bade 1975); and, finally, (7) Mu can integrate in both possible orientations within a given gene (reviewed in Howe and Bade 1975).

In further contrast to the λ-like integration/excision systems, Mu prophage is not induced by ultraviolet light or other agents known to induce λ prophage. Instead, exponentially growing cultures of Mu lysogens generally contain 10^5-10^6 plaque-forming units of spontaneously induced phage per milliliter, with some evidence suggesting that prophage derepression is caused by normal transcription of a Mu-infected DNA segment (Howe and Bade 1975). Even though Mu prophage induction occurs spontaneously at a moderate level, in an apparent contradiction, reversion to prototrophy is practically undetectable. Why? Howe and Bade (1975) correctly reasoned that this low reversion frequency might be because prophage are excised infrequently and/or excision usually results in cell death. The isolation and experimental use of temperature-sensitive Mu mutants, called Mu*ts*, which can be induced at 42°C to excise from the prophage state and are probably affected in a prophage repressor gene, have been instrumental in solving this problem. For example, utilizing *E. coli* lysogenized in the *lacZ* gene with a temperature-inducible Mu mutant, Bukhari (1976) has isolated, upon Mu induction, non-conditional phage mutants, called *X*, that are unable to replicate their DNA or express other lytic functions, but which allow for Mu excision and subsequent *lac* gene expression. Precise excision of induced Mu*X*, *ts* prophage resulting in Lac^+ revertants occurs at a frequency of 10^{-6}, while imprecise excision occurs ten times more often and yields defective revertants that remain $LacZ^-$ but which express the more operator-distal *lacY* gene. Precise reversion of Mu*X*, *ts*-induced bacterial mutations, although infrequent, demonstrates in these instances that Mu is inserted without the alteration of any bacterial sequences adjacent to its insertion site and suggests that integration/excision involves specific recognition of sequences at the Mu *termini* (Bukhari 1976). The recent isolation of deletion mutants of Mu that have lost the terminal sequences on one Mu end and which fail to lysogenize, lend support to the concept of specific terminal recognition sequences (Bukhari 1976; Chow and Bukhari 1977). Phage genes A and/or B, which are thought to be involved in integration and replication, respectively, also appear to be required for other Mu-promoted recombinational events such as deletion formation and chromosomal genetic inversion or translocation (Faelen et al. 1977, 1978;

O'Day et al. 1978). Mu phage growth, following induction of a thermoinducible lysogen or infection of *E. coli* cells with Mu, appears to require the host *dnaC* replication initiation function, the host DNA elongation factor encoded by *dnaB*, and host DNA polymerase III (Toissant and Faelen 1974). Aside from these replication requirements, no bacterial genes have been identified that are absolutely required for the various Mu-promoted recombinational events. However, some of the bacterial host mutations that inhibit λ integration also inhibit Mu induction and growth, and probably these host genes code for common DNA metabolic proteins that are necessary for a variety of processes (Kleckner 1977; Miller and Friedman 1977).

b. Molecular organization

Physical analysis of Mu phage DNA has revealed several unexpected complexities (see Fig. 7a and reviews by Howe and Bade 1975; Bukhari 1976; Couturier 1976). Mu is packaged as a linear, double-stranded DNA molecule of slightly variable size averaging 25 megadaltons (i.e., ∿37,000 nucleotide base pairs or ∿37 kilobase pairs). Since Mu DNA contains neither detectable cohesive ends nor terminal redundancy, it lacks any obvious means to circularize (Couturier 1976). DNA molecules released from Mu phage that were isolated from a single plaque have been found to vary in size from about 36-38 kilobase pairs (Martuscelli et al. 1971; Daniell et al. 1973a,b). When the phage DNA molecules originating from a single plaque are completely denatured and allowed to reanneal, structures like those depicted in Fig. 7b and c are observed. The resulting molecules are predominantly double-stranded and generally contain variable length (0.5-3.2 kilobase pairs) heterogeneous terminal sequences represented by the long single-stranded (split) ends at one terminus (Fig. 7b,c). Short variable length sequences of 100 base pair average size have been identified at the opposite Mu terminus more recently (see Chow and Bukhari 1977). Additionally, some reannealed molecules contain an internal 3.0 kilobase pair non-renaturable region (generally termed a substitution bubble) called the G segment, which is located at a constant position within these molecules and always proximal to the longer split ends. The cogent features of Mu DNA that have emerged from various molecular and genetic analyses (see recent reviews: Bukhari 1976; Couturier 1976; Chow and Bukhari 1977) have been summarized below and in Fig. 7a. The heterogeneous terminal regions of Mu are comprised of seemingly random bacterial sequences that differ among phage molecules. The physical map of Mu has been divided into the α, G, and β segments, as shown in Fig. 7a. Prophage immunity functions map close to one end of Mu (now termed the immunity end) and lie adjacent to the majority of known Mu genes, which are located in the 31 kilobase pair α segment. The 3.0 kilobase pair G segment frequently undergoes genetic inversion so that this region is distinguished as an internal substitution bubble in some reannealed phage molecules (Fig. 7c). The remaining 1600 base pairs of actual Mu DNA, the β segment, is situated immediately adjacent to the long heterogeneous terminal sequences, referred to as the variable end (Chow and Bukhari 1977). Therefore, disregarding the

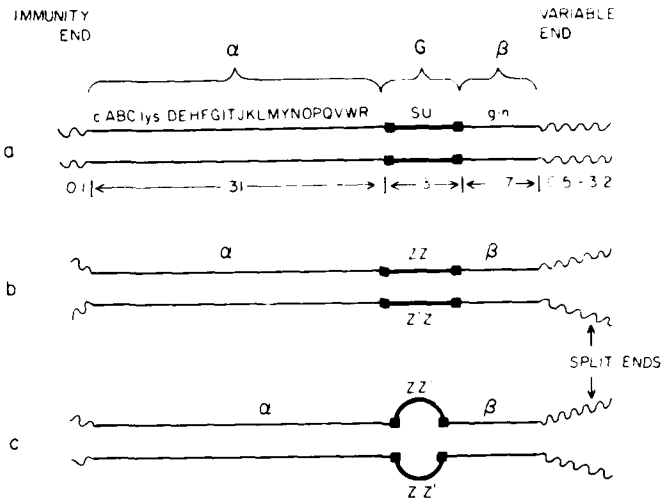

Fig. 7. Physical structure of Mu molecules. Each horizontal line represents a single-strand of phage DNA. (a) Linear double-stranded mature phage DNA molecule of approximately 25 megadaltons (not drawn to scale). The heterogeneous terminal sequences, represented by a squiggle line, consist of random bacterial sequences that vary in composition and length from molecule to molecule. True phage DNA sequences are divided into α, G, and β segments. The size in kilobase pairs for each region is given (Chow and Bukhari 1977; Kahmann et al. 1977). The invertible G segment is bounded by small (~20 base pair) inverted repeat DNA sequences represented by solid blocks, which are thought to be involved in the inversion of this segment. The middle one-third of the G segment contains a less stable pair of inverted repeat DNA sequences, identified in the electron microscope, that are represented in steps b and c as "Z'Z'". The map order of known genes on Mu phage is shown in (a) Bukhari et al. 1977; M. Howe, personal communication). The immunity gene c, integration gene A, and replication gene B, are located at one end of Mu, termed the immunity end. Genes S and U are located in the G segment, the inversion of which is controlled by the gin function that is encoded in the β segment. (b,c) Double-stranded heteroduplex structure resulting from the reannealing of different denatured Mu molecules. One end of reannealed Mu molecules, termed the variable end, was observed to contain long heterogeneous terminal sequences which have been referred to as split ends. More refined techniques have also revealed the existence of short heterogeneous terminal sequences at the immunity end. In the molecule shown in (c), the G segment is inserted in opposite orientations in each strand, creating a substitution bubble

heterogeneous termini, the actual phage DNA sequences in all Mu molecules are identical barring only the inverted G segment in some molecules.

A. Integration/Excision

Electron microscope and DNA sequence analyses of plasmids or phages harboring a Mu prophage have shown that none of the heterogeneous terminal sequences associated with autonomous Mu molecules is inserted during integration (Hsu and Davidson 1974; Allet 1978). It seems reasonable at this point to assume that Mu somehow sheds its terminal host sequences prior to or during integration. Randomly isolated Mu*X*, *cts* prophage-mediated auxotrophs have been observed to revert to prototrophy, probably by precise prophage excision, indicating that most Mu prophage exist as point insertions (Bukhari 1976). Furthermore, since Mu prophage and phage maps are collinear, this implies that insertion requires the specific recombinational interaction between the true Mu termini. One might logically hypothesize, then, that the mechanism of Mu integration is similar to that of λ or P22, except that the bacterial *att*Mu receptor sites are numerous and the attachment/recognition sequences on Mu are located immediately adjacent to the heterogeneous host sequences present in mature phage, as illustrated in Fig. 8. Unlike cohesive-ended λ or terminally redundant P22, however, Mu molecules have no obvious physical means to circularize, a requirement for the chromosomal integration of many phages and plasmids (Campbell 1976). As expected, infecting Mu molecules have never been observed to form covalently sealed circles (Bukhari 1976). However, the predominant Mu form observed after infection sediments in neutral sucrose gradients twice as fast as linear Mu monomers. This faster sedimenting form might represent a Mu DNA-protein complex, similar to that shown in Fig. 8B, where a protein accomplishes the non-covalent fusion of the true Mu termini. Recent evidence indicates that intracellular infecting Mu phage are assimilated by the host very slowly, and that Mu integration appears to require both Mu and bacterial DNA replication (Ljungquist et al. 1979). Perhaps the infecting Mu molecule acts as a template upon which only the true Mu phage sequences are replicated; this newly replicated single- or double-stranded structure might form short-lived covalently sealed circles or, possibly, undergo fusion of the Mu termini through the assistance of a protein (see Fig. 8B). Regardless of the exact intermediate, Mu integration apparently involves a specific recombinational exchange between the Mu termini and the host receptor site, resulting in linear Mu insertion, as illustrated in Fig. 8D. The gene *A*-encoded integration function and the terminal recognition sequences are the only presently known phage functions or sites needed for Mu integration and possibly excision (Bukhari 1975; Faelen et al. 1978; O'Day et al. 1978). Although infrequent, precise prophage excision can be detected in induced Mu*X*, *cts* lysogens. Restoration of the original host sequences at the receptor site might involve essentially a reversal of the integration process, i.e., recognition of the Mu termini by phage-encoded functions (Fig. 8C,D). However, recent DNA sequence analyses have revealed that a 5 base pair bacterial sequence at the insertion site is apparently duplicated in direct order during insertion, so that a 5 base pair repeat sequence flanks the inserted phage (Allet 1979). Thus, reversion of a Mu prophage-induced mutant would necessitate excision of one 5 base pair repeat plus the entire

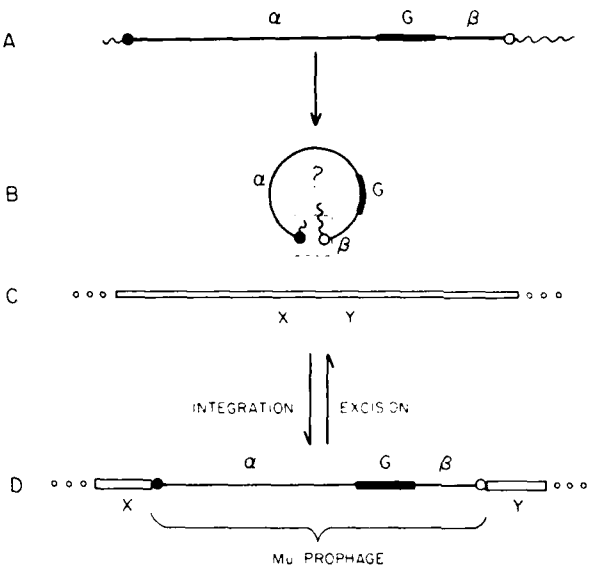

Fig. 8. Hypothetical illustration of precise integration and excision of Mu. (A) Linear double-stranded Mu DNA molecule as packaged in the virion. The heterogeneous bacterial DNA associated with each mature phage molecule is represented by *squiggled lines*. The true phage sequences α, β and G, are shown as *solid lines of different width*. The Mu attachment/recognition sequences are indicated by the *open and filled circles*. (B) Presumptive integration intermediate structure in which the true ends (i.e., *att* sites) of Mu are brought within close proximity (enclosed within *dashed lines*), perhaps by covalent linkage or via protein. The infecting Mu phage might undergo replication only of its true Mu sequences (i.e., excluding the heterogeneous ends) and this newly replicated structure (single- or double-stranded) could be the integration intermediate. (C) Portion of a bacterial, plasmid, or phage recipient chromosome, represented by an *open rectangle*. (D) Physical integration of Mu results in the linear insertion of only the true α, β, and G segments of Mu into the recipient chromosome. Although Mu can be inserted in either of two physical orientations, the prophage and vegetative phage maps are always collinear. Precise excision of Mu may simply involve the reversal of the integration process (discussed in detail by Bukhari 1976; Ljungquist et al. 1979; Couturier 1976) or may be mediated by some Rec-independent bacterial recombination process, as described in the text

prophage, an event that may be mediated by some bacterial process which recognizes short, directly repeated sequences (see Sect. C.V.). Relative to λ phage specialized recombination, our level of understanding of Mu integration/excision is very elementary. Furthermore, Mu phage induction constitutes a biological paradox as described below.

If only true Mu sequences are chromosomally inserted during prophage formation, the question arises, how do the phage particles isolated from an induced single Mu lysogen acquire many different terminal host sequences? The observations that Mu··· prophage can be induced independently of host Rec-ability and that Mu does not encode a general genetic exchange system (see Howe and Bade 1975) would lead one to believe that the heterogeneous phage termini are generated during replication and/or packaging, but not via recombination. Remember that in bacteria lysogenized by various other temperate viruses (e.g., λ, P22), induced prophage in virtually every cell excise and replicate as autonomous units. However, upon Mu··· prophage induction, only one out of every 10^5 cells appears to lose the prophage by exact or inexact excision, as detected by mutant reversion or loss of polarity, although every prophage is seemingly induced. Assuming that this situation closely mimics wild-type Mu induction, then most induced Mu prophage do not excise from the original integration site, yet they are replicated and give rise to progeny phage containing heterogeneous terminal sequences. This is the Mu paradox! Apparently, an induced Mu prophage generally replicates in situ at the original site of integration and transposes either single or double-stranded replicas of itself to other chromosomal loci where they insert, probably via the Mu integration system (Ljungquist and Bukhari 1977; see Fig. 10b,d). The newly inserted prophage can continue the lytic replication/transposition process giving rise to multiple lysogens containing ten or more Mu prophages (Toussaint et al. 1977; Ljungquist et al. 1979). In striking contrast to other temperate phages' integration functions which are not utilized during lytic growth, Mu integration functions (e.g., gene A) are necessarily expressed during productive lytic growth (Bukhari 1976).

Completion of the unique Mu lytic cycle encompasses encapsulation of mature phage, cell death, and virion release (Howe and Bade 1975). Compelling evidence indicates that Mu is packaged by a headful mechanism with encapsulation beginning at the phage immunity end. It is not currently known if the mature phages are excised and packaged directly from the host chromosome, from the large supertwisted circular molecules of varied sizes comprised of both Mu and host DNA that have been observed during lytic growth (see legend Fig. 10k), or in some alternate manner (Bukhari 1976, 1977; Waggoner et al. 1977). However, the packaging enzyme responsible for generating the immunity end of mature Mu molecules apparently recognizes a specific Mu site, but cleaves at a variable distance from the recognition site, averaging about 100 nucleotides into the adjacent host sequences (similar to the action of Type I restriction endonucleases). Beginning with the immunity end of Mu, the entire phage is packaged along with 500-3200 base pairs of adjacent bacterial DNA which is attached to the opposite Mu terminus. Recent experiments have shown that Mu phages containing sizable internal deletions or additions are packaged with longer or shorter stretches, respectively, of bacterial DNA attached to the ··· end of the phage molecules (Chow and Bukhari 1977; Chow et al. 1977). Therefore, the heterogeneous Mu termini seemingly result from the encapsulation of Mu molecules that are inserted within many different host sequences.

4. Inverted DNA Inversion Regulates Phage Viability

The G segment of Mu has the remarkable and somewhat perplexing ability to undergo *recA*-independent inversion. This relatively high frequency inversion event is thought to occur by recombination between short inverted repeat DNA sequences (~ 20 base pairs in length) found at the ends of the G region (Hsu and Davidson 1974; Chow and Bukhari 1977). The importance of these events is just being realized. Recently, a Mu gene (termed *gin*, for G inversion) that controls G inversion and which probably encodes a recombinational enzyme has been mapped in the *c* segment (Chow et al. 1977). Further studies have shown that in monolysogens a Mu *gin*$^-$ prophage with either G orientation appears to be produced equally well upon induction, but that only mature phage containing G in one specific orientation, the lytic orientation, can successfully infect similar bacterial hosts (Kamp et al. 1978). Several lines of evidence suggest that phage-coat borne adsorption functions, encoded by genes *S* and *U* which are located on the G segment, are not expressed when G is in the opposite orientation and, thus, resulting phage can not adsorb to similar host cells. However, Howe (1978) has intuitively noted that there may be two sets of adsorption functions on the G segment, each on opposite DNA strands and each of which express different adsorption proteins. Thus, G segment inversion may act as an on-off switch controlling phage viability, as supported by present data, or G inversion may change the host range of Mu.

It is very noteworthy that phages D108, P1, and P7 contain an invertible sequence that is virtually identical, by heteroduplex analysis, to the Mu G segment (Hull et al. 1978; Kamp et al. 1978, 1979). P1 and P7 are similar temperate viruses, which are circularly permuted and terminally repetitious, that exist as plasmids in the prophage state and, in most respects, are very different from Mu. Moreover, in P1 and P7 the G segment is bordered by larger (620 base pair) inverted repeat sequences (Chow et al. 1978b). However, G segments in Mu *gin*$^-$ phages can be inverted in the presence of P1 phage, demonstrating functional relatedness between these inversion systems (Chow and Bukhari 1977). The lack of homology between Mu and P1 or P7 suggests that these invertible G segments are capable of independent translocation from one molecule to another and may have arisen in these diverse systems by such an event (see Howe 1978). More important thant origin, what is the significance of genetic inversion to the phage or host bacterium? Recently, Zeig et al. (1978) have reported an inversion event that controls the alternating but exclusive expression of one of two flagellar antigenic types in *Salmonella*. In conjunction with these data, G inversions in phage demonstrate that inversion of DNA segments is a general mechanism, at least in bacteria and their viruses, for the control of gene expression (mechanism discussed in Sect. C).

5. Mu-promoted "Illegitimate" Genetic Exchanges

As discussed above and outlined in Table 2, Mu phages participate in what appears to be a variety of specialized recombination

Table 2. Specialized recombination events involving Mu phage

Type of event	Functions and sites		Physical state of phage	
	Required	Not required	Initial	Final
Phage integration	Mu *A*, *att* sites	Mu *B*	Infecting autonomous phage with random bacterial sequences at its terminal	Prophage in either orientation at any site of a recipient genome. The random bacterial sequences attached to mature Mu are lost upon integration
	(host replication functions)[a]	host *recA*, *recB*, *recC*, *recF*		
Phage transposition	Mu *A*, *B*, *att* sites	-	Replicating prophage	Multiple prophages with Mu in either orientation at two or more chromosomal loci
	?	host *recA*		
Precise prophage excision	Mu *A*	Mu *B*	Prophage	(Autonomous phage without heterogeneous termini)
	?	host(*recA*), *recB*, *recC*, *recF*		
Imprecise prophage excision	Mu *A* (Mu packaging enzymes)	Mu *B*	Prophage	Autonomous phage with heterogeneous termini
	?	Host(*recA*)		
G segment inversion	Mu *gin*, specific G segment termini	-	Prophage with G segment in either orientation; orientation of G regulates phage viability	Unaltered
	-	host *recA*, *recB*, *recC*		
Mu-promoted chromosomal deletion	Mu *A*, *att* sites	Mu *B*	Prophage or infecting phage	Prophage
	-	host *recA*		
Mu-promoted chromosomal inversion	Mu *A* or *gin* *att* or G segment termini	-	Prophage or infecting phage	Two prophages, separated by the inverted DNA segment, that are in opposing orientations or that have G segments in opposing orientations
	-	host *recA*		

Final state of recipient chromosome	Frequency	References
Insertion mutation, i.e., the Mu prophage	1-10% of the cell survivors of a single cycle of Mu infection are lysogens	Howe and Bade (1975) Couturier (1976) O'Day et al. (1978) Ljungquist et al. (1979)
Multiple lysogen	Occurs apparently for all prophages following induction	Toussaint and Faelen (1974) Bukhari (1976) Faelen et al. (1978)
Original host sequences restored upon prophage excision	Detected only upon induction of Mu *X*, *cts* mutants at 10^{-8} -10^{-6} events/cell	Bukhari (1975, 1976) Couturier (1976)
Chromosomal deletion; entire prophage and adjacent bacterial sequences are deleted together	Detected only upon induction of Mu *X*, *cts* mutants at 10^{-7}-10^{-5} events/cell	Bukhari (1975, 1976) Couturier (1976) Toussaint et al. (1977)
Unaltered	50% of the Mu released from an induced Mu *cts* lysogen have G segment inverted	Daniell et al. (1973b) Howe and Bade (1975) Kamp et al. (1978, 1979)
Lysogenic and containing deletions of host sequences that were originally adjacent to prophage insertion site	10-15% of Mu lysogens contain adjacent deletions	Howe and Bade (1975) Toussaint et al. (1977) Faelen et al. (1978)
The inverted host DNA is flanked on each side by a Mu prophage	10^{-4} events/cell in induced Mu *X* lysogen	Faelen et al. (1978) Toussaint (personal communication)

Table 2 (continued)

Type of event	Functions and sites		Physical state of phage	
	Required	Not required	Initial	Final
Mu-promoted integration of circular DNA	Mu *A*, *att* sites	Mu *B*	Infecting phage, or prophage, in the host or plasmid chromosome	Two prophages with same orientation, flanking the inserted DNA
	Circular DNA molecule	host *recA*		
Mu-promoted transposition of chromosomal DNA	Mu *A*, *B* *att* sites	-	Prophage or infecting phage	Two prophages with same orientations, flanking the transposed sequences
	-	host *recA*		
Internal Mu deletion	?	?	Prophage	Prophage containing internal deletions of up to 78% of Mu sequences. Resulting phage can be defective or viable
	?	?		

Functions or sites required or non-essential for these events are listed with the Mu functions/sites above the host functions/sites for each type of event. Although not required, Mu *B* increases the frequency of phage integration (Toussaint, personal communication)

Information shown in parentheses is conjectural

att = specific Mu termini

[a]host *dnaA* is not required, but host *dnaB*, *dnaC* and *dnaE* are required for Mu replication, which may preceed some of the above events

Final state of recipient chromosome	Frequency	References
Infecting phages promote plasmid integration at random chromosomal loci. In induced Mu lysogen, the plasmid is integrated at the original prophage site	10^{-4} events/cell in induced Mu *X*, *cts lysogens*	Howe and Bade (1975) Faelen et al. (1975) Toussaint et al. (1977) Faelen et al. (1978)
Transposed sequence flanked by 2 prophages and inserted into a new chromosomal locus	For any given gene, $2 \cdot 10^{-4}$ events/cell in induced Mu *cts* lysogen	Toussaint et al. (1977) Faelen et al. (1978)
Unaltered	?	Chow et al. (1977) Faelen et al. (1978)

events. Mu phage can insert as a discrete unit in either of two physical orientations within practically any locus, be it on a plasmid, phage or bacterial chromosome. However, due to the in situ replication/transposition lytic process characteristic of induced Mu *cts* prophages, reversion of Mu-induced mutations occurs only infrequently. Be that as it may, both precise and imprecise excision of Mu *X* prophages have been detected and these events require at least Mu gene *A* function (Bukhari 1975; 1977; Toussaint, personal communication). Also, phage viability or host range is controlled by the orientation of the internal invertible G segment relative to the surrounding Mu sequences. Mu integration/transposition and G loop inversion events are mediated by specialized recombinational processes that are encoded by Mu. Mu prophage excision involves an as yet uncharacterized recombination event which appears to be enhanced by the presence of *gpA* protein (Bukhari 1975).

As a consequence of the ability of Mu phage to replicate in situ and continuously transpose to different chromosomal sites during lytic growth, Mu causes a variety of aberrant host chromosomal rearrangements that ordinarily do not occur or which are detected at a much lower frequency in the absence of Mu (Toussaint et al. 1977, see Table 2). These events, all of which can occur in the absence of host Rec ability, include host chromosomal deletions, transpositions, and inversions, as well as the Mu-mediated chromosomal integration of autonomous circular DNA, Though the specific molecular mechanisms involved are not known, Mu gene *A* function together with the terminal Mu recognition/attachment sites are required to promote all of these events except Mu-mediated transposition of host sequences which apparently also requires the "replication" function specified by Mu gene *B* (Faelen et al. 1978; O'Day et al. 1978). Furthermore, besides these and possibly other Mu-encoded proteins, the Mu genome is a direct physical participant in both host Rec-independent and Rec-dependent chromosomal alterations. In addition to mediating host chromosomal rearrangements, Mu phage can undergo internal deletions entirely within the phage genome (Chow et al. 1977; Faelen et al. 1978). Therefore, besides promoting phage integration/transposition events, the Mu-mediated specialized recombination system(s) is involved in mediating macro-evolutionary chromosomal alterations, as described below and illustrated in Figures 9 and 10.

a) Deletions. Mu-promoted host chromosomal deletions can be generated by different mechanisms (see Table 2). Imprecise prophage excision results in the deletion of host sequences at the host chromosomal insertion site of Mu. This inexact excision of Mu prophages occurs at a relatively low spontaneous frequency (10^{-7}-10^{-5} events/cell) in a lysogenic bacterial population and involves the removal of the Mu DNA accompanied by some adjacent bacterial DNA sequences, frequently from both sides of the prophage insertion site (Bukhari 1976; Toussaint et al. 1977). Since there are 10^{5}-10^{6} viable phage per milliliter of an exponentially growing, non-induced culture of a Mu lysogen (Howe and Bade 1975), there must be a constant low level spontaneous induction of phage functions. Though entirely speculative, inexact Mu prophage excision may sometimes involve the premature packaging of chromo-

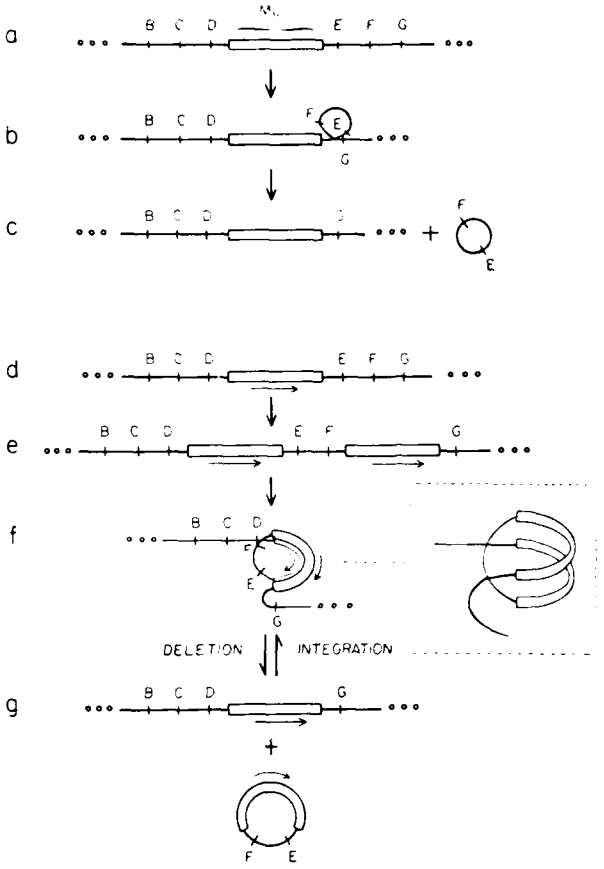

Fig. 9. Simplified hypothetical scheme for Mu-promoted deletion of host chromosomal DNA. (a) The *thin black horizontal line* represents a portion of a double-stranded bacterial chromosome which contains a Mu prophage, depicted by the *open rectangle*. Several hypothetical host genes are denoted alphabetically and the Mu prophage is inserted between host genes D and E. Many Mu-promoted host DNA deletions apparently occur from either Mu prophage terminus to random points within the adjacent chromosomal DNA and the prophage always remains intact. These Mu-mediated deletions might result from a recombinational exchange between either Mu terminus and nearby host sequences. (b) Deletion of host genes E and F could result from a reciprocal genetic exchange between a Mu terminus and a chromosomal site situated between genes F and G. (c) The resulting chromosome would contain a deletion of some host sequences immediately adjacent to one prophage terminus. The deleted material might exist as a non-replicating small circle which would be diluted out of the cell population during growth. Although this scheme allows one to conceptualize how Mu might mediate deletion formation, the more sophisticated model illustrated in Fig. 10 (b,h,k and l) is a more probable mechanism for this reaction.

somally linked prophage with concomitant closure of the host chromosome.

In contrast to these low frequency deletion events, Mu can promote deletions at much higher frequencies both during lysogenization or following partial induction of a thermoinducible prophage. Normally, during lysogenization about 15% of the inserted Mu prophage cause host chromosomal deletions that occur immediately adjacent to the prophage (see Howe and Bade 1975). Similarly, some of the survivors of partial induction of a Mu *ts* lysogen have been found to contain deletions of host sequences to either side of the prophage. In these instances, the prophage always remains intact and the deleted sequences span from either prophage end to a seemingly random point on the host chromosome. These recombinational events require the physical presence of a Mu prophage and can occur in the absence of host general recombination. It is probable that these latter events involve a common mechanism in which a Mu prophage(s) somehow undergoes an exchange between a Mu terminus and some nearby bacterial DNA sequence (Howe and Bade 1975; Toussaint et al. 1977). The mechanism of this deletion formation has not been elucidated. However, the simple reciprocal exchange between one Mu terminus and some adjacent bacterial sequences as illustrated in Fig. 9b,c offers, at least, a visual conception of this event. A less simple scheme proposed by Faelen and Toussaint (1976) is more likely to direct the deletions described above (see Fig. 10b,h,k,l). Their propo-

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(Legend to Fig. 9, continued)
It is important to note that although the above events do not require participation of the host Rec system, deletion formation can nevertheless occur between nearby Mu prophage via host chromosome-mediated general recombination. (d,e) Partial induction of a Mu monolysogen could result in the formation of a dilysogen. (f) Recombinational crossover between the nearby prophages could occur at any point along the paired Mu genomes. (g) In this example, general recombination between the identically-oriented, paired, nearby prophage resulted in the same chromosomal product as shown in (c). However, the deleted material now consists of all of the host DNA sequences initially located between the prophages (i.e., host genes E and F) as well as one entire Mu genome.

As shown in diagrams (g) to (f), the Rec-dependent integration of circular DNA into the chromosome can occur if both circular DNA's contain a Mu prophage. Though not shown, deleted host sequences like those depicted in step (g) could be reinserted at a new chromosomal prophage locus, an event that would constitute site-specific, Rec-dependent translocation of host DNA. Thus, Rec-dependent integration occurs by the apparent reversal of the deletion process and Rec-dependent transposition encompasses a deletion event followed by reintegration of the deleted sequences at a new chromosomal locus.

Imprecise prophage excision (not shown above) can also generate deletions of adjacent host sequences, frequently from both sides of the prophage insertion site. In contrast to the above events, the prophage is always removed during inexact excision. Although entirely speculative, the inexact excision may involve the premature packaging of chromosomally linked prophage

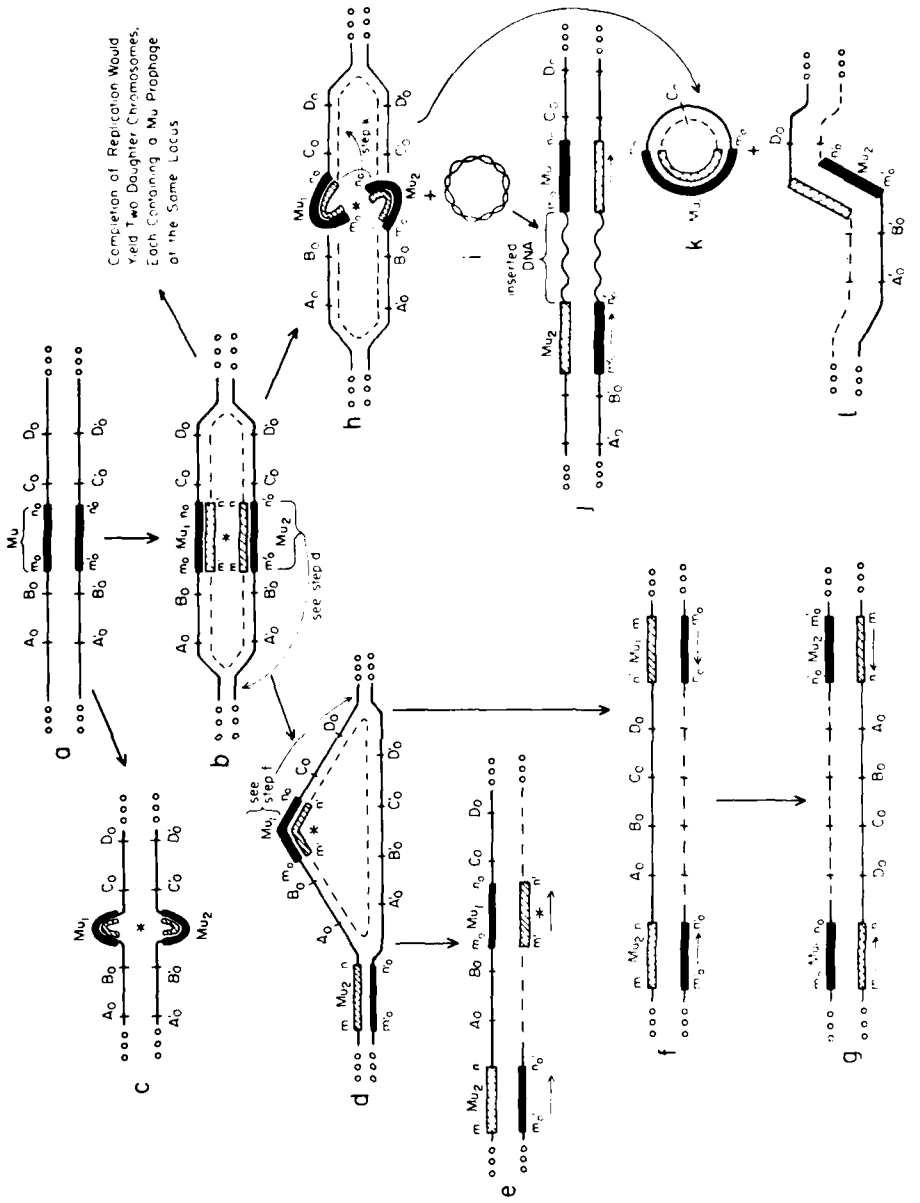

Fig. 10. Mu-mediated aberrant chromosomal rearrangements. Bacterial or plasmid single-stranded DNA sequences are represented by *thin horizontal lines* and Mu prophages are depicted by *rectangular boxes*. *Dark lines* or *narrow angles* with enclosed *slanted lines* indicate newly synthesized bacterial or phage DNA strands, respectively. Bacterial genes A, B, C and D, shown on one

(Legend to Fig. 10 continued)
DNA strand, and the complementary sequences (A', etc.) are labeled with *subscript zero* to indicate the initial sequences of the unreplicated parental molecule. Similarly marked are the specific Mu termini at the immunity and variable ends, which have been labeled *m* and *n*, respectively, in keeping with the terminology of Faelen and Toussaint (1976). As described below, most chromosomal rearrangements promoted by Mu can be explained on the basis of in situ prophage duplication coupled with subsequent prophage recombination events. The hypothetical scheme illustrated here is an adaptation and extension of the models previously proposed by Faelen and Toussaint (1976) and Toussaint et al. (1977). (a) A portion of bacterial chromosomal double-stranded DNA with a Mu prophage inserted between bacterial genes B and C. (b) During chromosome replication the segment carrying the prophage can be duplicated, giving rise to two prophages in opposite arms of the replication fork. Newly replicated Mu DNA appears to be the "active" form upon which the Mu integration function can act and these recombinationally active forms of Mu are denoted with an *asterisk*. Perhaps during growth, Mu DNA is specifically modified (e.g., by nucleotide methylation) at some point in both strands and the modified Mu cannot be attacked at the specific termini by the Mu integration enzyme. However, following semi-conservative replication of a Mu prophage, the integration enzyme might be able to recognize and act upon the termini of Mu that are modified in only one strand.

The prophages in (b) are depicted as having been replicated during normal chromosome duplication. Host-determined replication is sufficient to create recombinationally active prophages that can promote most genomic rearrangements. Completion of a round of replication would generate two daughter molecules, each containing prophages in the same location. In addition to Mu being replicated along with the host genome, specific Mu-determined prophage replication can not only occur, but is required for Mu-mediated transposition of host DNA. (c) Mu-specified replication of the Mu prophage would generate recombinationally active Mu prophage, which could also cause the same chromosomal rearrangements as shown in the following steps. However, the structures shown in (b) and (h) more easily convey a picture of the "recombinationally active", newly replicated, daughter Mu prophages. (d) This example illustrates the specific translocation of the lower prophage, Mu_2, shown in (b) to a point outside of the replication fork. Mu transposition might occur, as shown, by the recombination of the Mu_2 termini with a DNA sequence to the left of gene A. Although Mu could be inserted in either orientation, I have chosen to show a direct transposition. (e) Following either degradation of the replication fork from which Mu_2 was deleted or completion of a round of replication, the chromosome would be dilysogenic with both prophages in the same physical orientation as depicted by the *horizontal arrows*. It is important to recognize that recombinational activation of the Mu termini apparently accompanies prophage replication. However, Mu transposition rarely causes a reversion of the original Mu insertional mutation, evidently because only one daughter prophage is commonly transposed. Alternatively, transposition may occur by single-strand exchange and always leaves Mu at the original site. (f) In addition to the transposition event shown in (d), precise prophage excision might result from the subsequent transposition of the upper prophage, Mu_1 (also shown in d) to some other chromosomal site. In this case, Mu is transposed in inverted orientation, rightward to a point adjacent to the replication fork. Upon dissolution of one strand of the replication fork or completion of host genome replication, the chromosome would be dilysogenic with the two prophages in opposite orientations (f). As a consequence of precise prophage excision, the host sequences at the initial prophage insertion site, as

(Legend to Fig. 10 continued)
shown in (a), are restored. (g) Host sequences flanked by nearby prophages in physical apposition, as shown in (f), are substrates for inversion. This diagram illustrates the product of an inversion which occurred between the outer termini of the prophages. Inversion of the enclosed host and phage sequences has also been observed between the oppositely oriented G segments of two identically oriented, nearby prophages (Faelen et al. 1977; not shown).

"Active" Mu termini, although attached to random bacterial DNA during integration or transposition, can insert into virtually any chromosomal locus (see Fig. 8). Because of the proximity to each other of the newly duplicated prophages shown in (b), the integration enzyme complex may recognize the ″ end of one prophage together with the ′ terminus of the opposite prophage, and recombine these opposing ends as shown in the following steps. (h) This schematic shows the interacting opposite ends of the two prophages in a "fused" state, ready to recombine with any DNA sequence. In order to emphasize the interacting ends of the opposing prophages, one end of each prophage is illustrated as being disconnected from its corresponding arm of the replication fork. However, the interaction of the two opposing prophages may not initially require such disconnections. Toussaint, Faelen and coworkers have ingeniously deduced that "fused", newly replicated prophages, as shown in (h), can react with circular DNA to promote its insertion or can cause the deletion of adjacent host bacterial sequences (Faelen and Toussaint 1975; Toussaint et al. 1977). (i) A covalently closed, circular double-stranded DNA molecule, such as λ*dgal* or a bacterial plasmid, is represented by the *squiggled line*. (j) Linear insertion of the circular DNA in any permutation would occur by recombination between the "fused" prophage termini, shown in (h), and the circular DNA. The resulting chromosomal insertion would occur at the original prophage insertion site on the host chromosome and the inserted DNA would be flanked by identically-oriented Mu prophages. Note that similarly oriented, nearby prophages are susceptible to host Rec-dependent recombination and could result in deletion of one phage and the interposed host sequences, as shown in Fig. 9g. Instead of recombining with an autonomous circle, if the "fused" prophage termini (h) had recombined with some nearby chromosomal sequence, a site-specific deletion would be generated. (k,l) Recombination between bacterial sequences C_O and D_O would result in the deletion of a circle (k) containing one prophage, Mu_1, and the DNA sequences immediately surrounding C_O. Similar circular molecules have been observed following Mu prophage induction and may be the structures from which mature phage containing heterogeneous termini are obtained for virion packaging. Following the deletion of Mu_1 and bacterial gene C_O (h), chromosomal circularity would be reestablished by the joining of Mu_2 to a point close to the sequences labeled D_O (l). Exonucleolytic degradation of the $\sim(A_O\text{-}B_O)\text{-}$ and $\text{-}(C_O'\text{-}D_O')\sim$ arms of the replication fork (h) would result in a bacterial chromosome containing a deletion of host sequences occurring immediately adjacent to the remaining prophage, as shown in (l). Though not shown, recombination between the "fused" Mu termini (h) and a point outside of the replication fork would generate circles with tails, similar to those observed following prophage induction (Toussaint et al. (1977).

Transposition of host sequences might require two of the above described recombination events. First, newly replicated prophages could cause the deletion of a circle comprised of one prophage and some adjacent host sequences, as shown in (k) and (l). Secondly, specific Mu-determined replication of the prophage in this circle would generate a Mu-Mu structure similar to that depicted in (c). "Fusion" of the ″ and ′ termini of opposite

(Legend to Fig. 10 continued)
prophages and recombination with the host chromosome at any locus would result in the transposition of bacterial sequences. The transposed sequences would be flanked by identically-oriented Mu prophages just as shown in (j)

sal takes into account the currently known properties of Mu, especially its ability to integrate randomly, and the fact that Mu-mediated deletions do not require the Mu B "replication" function. Simply stated, host chromosome-determined replication of a prophage would generate two prophages on daughter arms of the replication fork, as shown in Fig. 10b. "Fusion" of these prophages into a structure resembling a directly repeated tandem Mu dimer would generate a "recombinationally active site", the adjacent Mu termini (see Fig. 10h). This "active site" could recombine with any nearby host chromosomal sequence, always resulting in the deletion of one Mu genome plus some adjacent host DNA and leaving one intact Mu prophage at the site of the deleted sequences (see Fig. 10k,l).

All of the above deletion events can occur via specialized recombination. However, it is very important to emphasize that due to the relatively large size of Mu DNA, host Rec-dependent exchange between Mu prophage can also generate host chromosomal deletions (Faelen et al. 1977). Spontaneous induction of a Mu prophage could generate identically oriented, nearby prophage which flank some host genomic sequences (see Fig. 9l). Rec-dependent random genetic exchange between paired, nearby prophage genomes (Fig. 9f) would result, like the phage termini-specific exchange described in Fig. 10k,l, in the deletion of one Mu genome and any interposed bacterial sequences (see Fig. 9g). Thus, the integration via specialized recombination of two or more Mu into nearby regions of the bacterial chromosome results in the prerequisites (i.e., large homologous DNA regions) for Rec-dependent recombination. In other words, specific DNA segments like Mu phage or transposons (see later section) which are recognized and exchanged by specialized recombination enzymes can also be substrates for homologous recombination systems.

Aside from mediating deletions in recipient chromosomes, Mu prophages have been observed to undergo internal deletions of Mu sequences. Recently, Faelen et al. (1978) have isolated, by thermal induction of Mu cts lysogens, mini-Mu mutants which contain large internal deletions of up to 78% of the Mu genome. These mini-Mu phage still carry the Mu terminal recognition/attachment sequences and can be propagated in the presence of helper phage. Furthermore, when induced these mini-Mu phage can transpose and promote chromosomal rearrangements. Another thermoinducible Mu lysogen, from which non-defective internal deletion mutants have been obtained, contains an unusual 2.6 kb insertion, which has been identified as insertion sequence elements IS1 and IS2 (Chow and Broker 1978). Although the specific mechanism of Mu phage internal deletion formation is not known, all such deletion mutants, to date, have been found to contain insertion sequence elements that may be responsible for these deletion events (see Sect. IV.2.b.). The involvement of Mu genes in the formation of these internal deletions is presently unknown (Chow et al. 1977).

b) Integration of Circular DNA. Host chromosomal integration of circular DNA molecules, such as *λdgal* or bacterial plasmids, can be mediated by Mu (Faelen et al. 1975; Howe and Bade 1975; Toussaint et al. 1977). This process, outlined in Table 2, can occur independently of host Rec ability, but requires Mu gene *A* function, as well as the physical participation of the Mu genome itself. Mu-mediated integration occurs at frequencies as high as one per 10^4 Mu *cts* phage in an induced population of monolysogens. The product is always the linear insertion within the recipient chromosome of the circular DNA molecule flanked on each side by one entire Mu genome, with both flanking prophages in the same orientation (Faelen et al. 1978). Considering that induced Mu *cts*, *B*⁻ (non-self-replicating) monolysogens have been observed to promote the integration of circular DNA (Faelen et al. 1975), how is the resultant second flanking prophage synthesized? Faelen et al. (1975) have proposed that two daughter Mu prophages, formed by normal chromosome replication of a preexisting prophage, can interact with one another to promote the integration of circular DNA, as illustrated in Fig. 10b,h-j. When integration is promoted by an induced Mu prophage in a monolysogen, the resulting "Mu-inserted DNA-Mu" chromosomal complex is always located at the original prophage insertion site. In contrast, infecting Mu phage can mediate the linear insertion of circular DNA into virtually any chromosomal site. However, in both cases the integrated DNA is inserted with any circular permutation. Although not described in Fig. 10, the above chromosomal integration event could also be mediated by a plasmid-borne Mu prophage. In this case, however, the duplicated daughter prophages, formed during plasmid replication, would promote the insertion of the plasmid with only one permutation into any host chromosomal locus.

Though Mu-mediated integration of circular DNA may occur by the process described in Fig. 10, an alternative mechanism has been proposed. Faelen and Toussaint (1976) previously theorized that infecting Mu phage might somehow dimerize by the fusion of two vegetative phage genomes. The resulting directly-repeated, tandem, Mu, circular dimer would contain two sets of hyperactive, fused Mu termini which could mediate the co-integration of two circles at random points. Finally, one should be cognizant of the fact that if both the bacterial chromosome and any circular DNA molecule simultaneously carry Mu prophages, Rec-dependent recombination between the paired prophages can promote chromosomal integration of the circular DNA (see Fig. 9q and legend).

c) Mu-mediated Transpositions. Mu phage has the remarkable ability to mediate the transposition of any DNA segment from one location to another on the same or a different molecule (see Table 2). Either an induced lysogen or an infecting phage can effect transposition of a chromosomal segment. The frequency of transposition for any specific chromosomal gene can be as high as 2×10^4 events/induced Mu *cts* lysogen (Faelen and Toussaint 1976). Assuming that the *E. coli* genome comprises about 3000 genes, then in an induced, Mu-lysogenic population a phenomenal one out of every two cells should contain a transposed DNA sequence. The end product is identical to that obtained in Mu-mediated plasmid integration, i.e., the transposed DNA segment is flanked by two

Mu prophages in the same orientation. Additional evidence indicates that: only closely linked markers are cotransposed; all genes situated between two cotransposed genes are simultaneously cotransposed; the cotransposition frequency of an unselected marker is related to its distance from the selected marker; and transposition of segments as large as 3.5 min of the *E. coli* chromosome or approximately 90 megadaltons (135 kilobase pairs) has been detected (Faelen and Toussaint 1976). Although transposition events, for all practical purposes, would be undetectable within a single cell, transposition of chromosomal genes has been readily assessed in cells carrying the F plasmid. Following induction of a cell population lysogenic for Mu *cts*, various segments of the host chromosome are transposed onto the F plasmid. Specific transposed genes can then be detected after conjugal plasmid transfer to suitably marked recipient cells. Unlike most other Mu-mediated specialized recombinational events which require only Mu *A* function in addition to the specific Mu termini, transposition of host sequences also requires the Mu gene *B* (presumptive replication) function.

Albeit the exact mechanism of Mu-mediated transposition has not been elucidated, Toussaint et al. (1977) have developed a model to explain this process. Accordingly, Mu-mediated transposition of host sequences is a two step recombinational process. Initially, newly replicated prophages interact, as shown in Fig. 10h, and promote the deletion of a circle composed of one prophage and some adjacent host sequences (see Fig. 10k,l). Subsequently, specific Mu-determined replication of the prophage in this circle (Fig. 10k) would generate a forked Mu-Mu structure similar to that illustrated in Fig. 10c. The second recombinational event would entail "fusion" of one ″ and one ′ terminus, each from opposite daughter prophages on the plasmid, and reciprocal genetic exchange of this recombinationally active site (i.e., the adjacent Mu termini) with the host chromosome at any locus. This process would result in the integration of the entire circle into the chromosome. The resulting transposed bacterial sequences would be flanked by identically oriented Mu prophages just as shown in Fig. 10j. Although newly transposed or integrated DNA segments are hereditarily stable in a Rec-deficient host, it is interesting to note that because of the large size of the flanking homologous Mu genomes, these insertions are deleted at a frequency of about 1% from Rec^+ hosts (Faelen et al. 1975; see Fig. 9e-g). In the absence of Mu specialized recombination systems, the host general recombination system may potentiate Mu-mediated transposition as described in the legend to Fig. 9. In contrast to specific transposition of the Mu genome that occurs following Mu induction and in which one Mu prophage generally remains at the original insertion site (Fig. 10d,e), Mu-mediated transposition of host DNA appears to involve the absolute deletion of host sequences from one area of the chromosome followed by their insertion into a different chromosomal locus. However, current evidence does not eliminate other explanations. Transposition of bacterial sequences that are flanked by directly repeated Mu prophages might occasionally involve recognition of the opposite ends of these prophages so that the recombinational exchange would mimic that observed in

specific Mu phage transposition in which one copy of the transposed segment always remains at the initial site.

DNA sequence transposition, in general, allows for the movement of relatively large, complex DNA segments to other chromosomal sites, sometimes placing them under different genetic regulatory controls. Also, transposition of large DNA segments to plasmid or phage vectors can promote rapid intercellular dissemination of hereditary information. Fusion of various genes to novel genetic promotors (Casadaban et al. 1977) and the in vivo cloning of a gene via Mu-mediated transposition onto a plasmid (Denarie et al. 1977; Faelen et al. 1977) have been successfully employed in recent genetic studies.

d) Mu-mediated DNA Inversions. Mu can mediate the inversion of adjacent chromosomal DNA sequences by two different mechanisms. Inversion in an induced Mu *λ* lysogen occurs at a frequency of 10^{-4}, requires Mu gene *A* product, and the inverted chromosomal sequence is usually flanked by two Mu prophages in opposite orientations (Toussaint, pers. commun.; Faelen et al. 1978; depicted in Fig. 10f,g). The prophages observed on both sides of the inverted host DNA sequence may not be a prerequisite for inversion, but may in some way be a consequence of the inversion process, as is the case for Mu-mediated insertion or transposition. Also, in addition to promoting G loop inversion, the Mu *gin* function can apparently promote inversion of all host and phage sequences located between two opposing G segments in nearby prophages (Faelen et al. 1977).

6. Effect of Mu on Bacterial Evolution

Temperate bacterial viruses are usually limited in host range to one or two genera because of limited bacterial species carrying the proper cellular surface receptors and/or the inability of the phage, once injected, to lysogenize or replicate. Though Mu will infect *E. coli* K-12, *Citrobacter freundii*, *Shigella dysenteriae*, and some strains of *Klebsiella pneumoniae*, Mu phage do not form plaques on *E. coli* C, B, S, or W, *Salmonella typhimurium* or other related enterobacteria. However, following integration of Mu into the conjugally promiscuous RP4 plasmid, this plasmid has been successfully employed to introduce Mu via conjugation into many different hosts. Recently, Mu has been observed to replicate, to lysogenize, and to promote chromosomal rearrangements in many diverse gram-negative bacteria which are not ordinarily susceptible to Mu infection (Denarie et al. 1977).

Temperate viruses that can physically integrate into the host chromosome are apparently each recognized by a specialized recombination system. Following virus integration as discrete DNA units at one (e.g., λ), a limited number of (e.g., P22), or many (e.g., Mu) chromosomal recognition/attachment sites, prophages can subsequently cause localized mutagenesis (e.g., host DNA deletion). Unlike other temperate phage, however, Mu and phage D108, which share most of their DNA sequences, can be inserted in either physical orientation at any chromosomal site, an event

causing either simple gene inactivation or a strongly polar mutation in an operon (Hull et al. 1978; Kamp et al. 1979). In addition, Mu specialized recombination systems mediate a variety of host genomic rearrangements. Therefore, Mu and λ may represent opposite ends of a spectrum of site-specific or specialized recombination systems, each of which recognizes a specific class of DNA elements (e.g., λ, insertion sequence elements, or Mu) and which can effect their specific insertion into only one, a few, or many recipient DNA sites. For the interested reader, many of the properties of Mu have been reviewed in detail elsewhere (Howe and Bade 1975; Bukhari 1976, 1977; Couturier 1976; Bukhari et al. 1977). One wonders how many more Mu-like viruses exist in nature. Certainly these viruses or their remnants within a chromosome must be responsible for a significant proportion of the genetic flexibility of many bacteria.

Recently, an increasingly large group of transposable genetic entities has been detected in many diverse bacterial genera. These elements do not appear to replicate in a physically autonomous state like a plasmid and cannot exist extracellularly like a virus, but they can promote a variety of host genetic alterations. As described in the following sections, these transposable elements appear to be functionally related to λ and Mu viruses in that all are recognized as discrete DNA units by specialized recombination processes.

IV. Transposable Genetic Elements

1. Definition

During the past decade, a variety of unique transposable DNA segments have been identified in the chromosomes of bacteria, their plasmids, and viruses. Apparently common constituents of chromosomes, these elements have been detected by their transposition to and inactivation of a known gene. Beyond their simple transposition to a chromosomal locus, these remarkable elements cause a variety of macro-evolutionary chromosomal rearrangements, similar to those promoted by the bacterial virus Mu. Furthermore, some of these transposable DNA units encode transcriptional initiation and termination signals and can act as supernumerary regulatory switches affecting gene expression (see reviews by Starlinger and Saedler 1972, 1976; Cohen and Kopecko 1976; Kleckner 1977; Starlinger 1977). The systematics and nomenclature of these elements have been summarized by Campbell et al. (1979).

These discrete DNA segments which range in duplex DNA length from about 750 to 80,000 nucleotide base pairs are structurally defined by repeated DNA sequences at their termini and are normally transposed intact as distinct, non-permuted units from one location to another on the same or a different molecule. No transposable element has yet been shown to replicate or to exist in a separate, physically autonomous state (i.e., they are al-

ways linearly inserted within a chromosome). As a probable consequence of their repetitious terminal sequences, these DNA units can insert with either of two physical orientations. In addition to sharing the common feature of terminal sequence repetition, all of these genetic elements are transposed independently of general recombination systems. Thus, these DNA segments appear to transpose via specialized recombination events. Transposable DNA elements have been identified, both genetically and physically, in a large variety of different bacterial genera, strongly suggesting their universal existence in bacteria.

It is necessary to define certain terminology before proceeding further. The exchange of a DNA segment between non-homologous chromosomes or non-homologous regions of the same molecule has been commonly termed either *transposition* or *translocation*. Though both words seem equally appropriate, the term *translocation* has also been used more recently by biochemists to describe the passage of molecules across a membrane or to denote the movement of peptidyl tRNA from the "A" to the "P" site of the ribosome during translation (Watson 1976). In order to avoid unnecessary confusion, it would appear expedient to refer to the exchange of a DNA segment between non-homologous DNA regions as *transposition*. For this reason, I have exclusively used the term transposition in the remainder of this review. The term *transposable elements*, originally used to define mobile genetic elements in maize (McClintock 1952), has been used colloquially to refer to all transposable genetic elements in eukaryotic and prokaryotic systems. However, in this section "transposable DNA elements" refers to a set of structurally defined prokaryotic DNA units that exist only in the integrated state. Transposition of a discrete transposable DNA element, presumably directed by specialized recombination, has been termed, variously, site-specific transposition, site-specific recombination, or simply genetic transposition. Though transposable elements are inserted as discrete units, their insertion has been observed to occur into many recipient chromosomal receptor sites and the term "site-specific" now seems too constrictive. Also, it should be strongly emphasized that transposition or deletion of specific chromosomal DNA segments can occur via general recombination (i.e., between nearby homologous DNA regions; see Fig. 7). Perhaps the general term "genetic transposition" should be used to refer to the in vivo movement of DNA from one site to another regardless of Rec-dependence. *"Specialized"* transposition might be employed to describe those events which are mediated by specialized recombination. Finally, the endproduct of most specialized transposition events appears to be different from Rec-dependent transposition. Like the replication/transposition process of an induced Mu prophage, specialized transposition of a transposable DNA element from one site to another does not cause loss of the transposable element at its original locus (i.e., transposition is not linked to precise excision). In other words, only a single DNA strand of the element or a newly replicated transposable segment appear to be substrates for specialized transposition. In contrast, as described in the legend to Fig. 9, general recombination-dependent deletion can occur between homologous DNA regions bracketing any interposed sequence. Subsequent Rec-dependent in-

sertion of this deleted material could potentially occur at any chromosomal region containing significant homology to the deleted circular molecule (see Fig. 9g). Likewise, Mu-promoted transposition of chromosomal sequences appears to involve Mu-mediated deletion of the sequences, followed by Mu-promoted insertion of these sequences into a new site (see legend to Fig. 10). Therefore, specialized genetic transposition of a specific transposable element does not appear to result in loss of the transposable element at its original site whereas in Rec-dependent or Mu-promoted transposition of chromosomal sequences, the segment to be transposed is apparently first deleted from one chromosomal site and subsequently inserted elsewhere.

Transposable elements have been divided into two classes, both for historical and structural reasons. The small insertion sequence (IS) elements, which were discovered in the late 1960's and do not encode any known phenotypically identifiable proteins, represent one class (Saedler and Starlinger 1967; Jordan et al. 1968; Shapiro 1969). The second group is exemplified by the relatively large transposable elements that encode resistance to various antibiotics and which were physically identified in the mid-1970's (reviewed by Cohen 1976; Cohen and Kopecko 1976). These large elements, seemingly more complex than IS units, are comprised of a central sequence bracketed by direct or inverted DNA sequence repeats, sometimes consisting of a bona fide IS element. More recently, genes encoding toxin production and a variety of metabolic capabilities have been identified on these larger transposable elements. Although this classification scheme for transposable elements is tenuous, as you will see, it does correctly emphasize the hierarchy of structural complexity in transposable elements. Regardless of complexity, however, all of these discrete elements are responsible for a large proportion of chromosomal rearrangements in bacteria.

A. IS Elements

Analysis of the fine-structure organization of information in DNA was carried out during the 1960's through the genetic and biochemical examination of both spontaneous and induced chromosomal mutations. A considerable fraction of the spontaneous mutations studied in bacterial or phage genomes proved to be quite unusual. Unlike base substitution mutations, these novel mutations exerted a very strong polar effect on the expression of more promotor-distal genes in an operon, similar to the effect of frameshift mutations. Reversion of these unusual mutations was not enhanced by mutagens that normally cause base substitutions or frameshift mutations. Moreover, these unusual mutations reverted spontaneously to wild type, ruling out nucleotide deletion and suggesting insertion or inversion as the original alteration. Comparison, by a variety of physical measurements, of parental lambda transducing phages to mutant lambda transducing viruses containing different novel polar mutations indicated the presence of a sizable insertion in each mutant virus and essentially eliminated genetic inversion as the cause of these mutations (reviewed by Starlinger and Saedler 1976).

By DNA-RNA hybridization studies, Michaelis et al. (1969) showed that the inserted sequences in these lambda mutants were not DNA sequence duplications, but rather DNA unlike the wild-type sequences. These insertion mutations which were later measured for length in the electron microscope and for homology with one another by the DNA heteroduplex procedure (Fiandt et al. 1972; Hirsch et al. 1972; Malamy et al. 1972), are now known to comprise several distinct classes (Table 3). Therefore, these novel mutations in *E. coli* and lambda were shown to be caused by a few distinct IS elements. Starlinger and Saedler (1972, 1976) have composed excellent reviews that explore the genetic and initial physical characterization of IS elements. As discussed below, these elements have now been characterized by a variety of genetic and biochemical methods.

a) Some Genetic and Molecular Properties. The general properties of IS elements are summarized below and in Table 3. IS elements are DNA segments that range in length from 768 to about 1400 nucleotide base pairs or more and are found in bacterial, phage and plasmid chromosomes. These disrete units can be transposed in either of two physical orientations as a linear, non-permuted unit to a recipient chromosomal site on the same or a different molecule. IS elements are usually detected by the characteristic effects that they exert at new sites, i.e., abolition of the function of a gene and alteration of the expression of promotor-distal genes in an operon (i.e., polarity).

Regarding *E. coli* and its plasmids and viruses, insertion mutations occur in many different locations of these genomes (e.g., at multiple sites within different genes in the well-studied *E. coli lac* or *gal* operons). Thus, statistically speaking, the recognition sequence(s) on the recipient molecule must be fairly short, as opposed to a longer sequence which would be duplicated less often around the chromosome. For example, a specific sequence of five nucleotides should recur on a random basis about every 1000 bases. However, it should be noted that for as yet undetermined reasons, certain chromosomal regions appear to be preferred areas for insertion. For example, in separately isolated mutants, IS elements have inserted at very close (i.e., several nucleotides apart) and at identical sites within the 200 base pair *gal* operon control region (Saedler et al. 1972; Kuhn et al. 1979). It appears that IS element integration is neither entirely random like the integration of Mu phages, nor as specific as the integration of bacteriophage λ into the primary *att*B locus. However, though IS elements appear to have a preference for certain DNA regions, insertion in different mutants occurs at many nearby sites within these defined areas (i.e., receptor site clusters) and also at many other sites around the genome. Thus, unlike the case of λ which, when the primary *att*B site is deleted from the bacterial genome, integrates into several different, but specific secondary *att*B sites, IS units express what has been termed "regional" specificity, i.e., the ability to integrate as a discrete unit in many sites within a preferred short recipient DNA segment.

Table 3. Insertion sequence elements in bacteria

IS element designation	Total Tn Length (b.p.)	Length and orientation of internal terminal repeat sequences (b.p.)	Restriction enzyme suseptibility	
			Cleaved by	Not cleaved by
IS*1*	768	28 of first 34 b.p. form inverted repeat	*Alu*I *Bal*I *Hae*II, III *Hha*I *Hin*fI *Hpa*II *Hph*I *Pst*I	*Bam*HI *Bgl*I, II *Eco*RI *Hinc*II *Hin*dII, III *Hpa*I *Xba*I *Xho*I
IS*2*	∿1327	32 of first 41 b.p. form inverted repeat	*Ava*I *Bgl*I *Hae*II, III *Hin*dII, III *Hin*fI *Hpa*II *Hha*I *Mbo*I *Sma*I *Taq*I	*Eco*RI *Bam*HI
IS*3*	∿1200	?	*Hin*dIII *Pst*I	*Bam*HI *Eco*RI *Hin*dII
IS*4*	1400	16 of first 18 b.p. form inverted repeat	*Ava*I *Hin*dII	-
IS*5*	1250	<50 b.p., inverted	*Eco*RI	-
γ-δ (Tn*1000*)	∿5800	35 b.p., inverted; terminal 28 b.p. identical to Tn A termini	*Eco*RI *Bam*HI *Hin*dIII *Kpn*I *Pvu*I *Sal*I *Sma*I *Sst*I *Xho*I	*Xba*I
IS*10*	1400	10 b.p. inverted repeat located 13 b.p. in from one end	*Acc*I	-

Effect of IS element orientation on transcription	Recipient genomes carrying IS units	Length of recipient DNA duplicated at ends of IS elements (b.p.)	Pertinent references
Polar in orientation I or II	*E. coli* *S. typhimurium*; *Citrobacter*; Phage P1, Mu, λ, T4; Plasmids R1, R6, F, R100, Ent	9	Jordan et al. (1968) Saedler and Starlinger (19[illegible]) Starlinger and Saedler (1976) Grindley (1978) Ohtsubo and Ohtsubo (1978) Hu et al. (1975a) Shapiro (1969) Fiandt et al. (1972)
Polar and contains rho-sensitive site in I and II; Promoter function observed in variants of I or II	*E. coli*; Phage λ; Plasmids F, R6, R100	5	Saedler and Heiss (1973) Saedler et al. (1974) DeCrombrugghe et al. (1973) Ghosal and Saedler (1978) Ghosal et al. (1979a,b) Pilacinski et al. (1977) Hu et al. (1975)
Polar in I; II not yet studied	*E. coli*; Plasmid F	3 or 4	Malamy et al. (1972) Hu et al. (1975b) Deonier et al. (1979) Sommer, Cullum and Saedler (personal communication)
Polar in I & II	*E. coli* *galT*	11 or 12	Habermann et al. (1979) Pfeifer et al. (1977)
Polar in I & II	*E. coli*; Phage λ		Blattner et al. (1974) Chow and Broker (1978)
?	*E. coli*; Plasmid F	5	Guyer (1978) Broker et al. (1977a) M. Guyer (personal communication)
?	Plasmids R6, R100	9	Kleckner (1979) Kleckner and Ross (1979)

Table 3 (continued)

IS element designation	Total Tn Length (b.p.)	Length and orientation of internal terminal repeat sequences (b.p.)	Restriction enzyme suseptibility	
			Cleaved by	Not cleaved by
"ISR*1*"	1100	-	*Bam*HI *Hin*dIII *Pst*I	-
Unassigned	500 - 1800	-	-	-

The first five IS elements have been convincingly demonstrated to undergo specialized transposition and precise excision in *E. coli*, as has the much larger γ-δ sequence, also termed Tn*1000*. Very recently, the 1400 b.p. repeat at the ends of the Tn*10* transposon has been shown to behave as an IS element and has been named IS*10* (Kleckner and Ross 1979). Also, an IS element has been physically and genetically characterized in *Rhizobium* and has been tentatively termed "ISR1". The variety of elements that are inferred from genetic data or visualized in the electron microscope as small inverted repeat DNA sequences, but which have not been proven to transpose are listed in the "unassigned" category. The DNA sequences of IS*1* and IS*2* have been analyzed entirely (Grindley 1978; Ohtsubo and Ohtsubo 1978; Ghosal et al. 1979b; Johnsrud 1979). Total IS element length is given in nucleotide base pairs (b.p.). If known, the length of the internal short sequence that is repeated in inverse order at the termini of some IS elements is also given. The physical orientation of each IS element within a chromosome, detected by physical and genetic means, has been arbitrarily designated I or II. The effect on promoter-distal gene expression by IS elements, when inserted in an operon, is given for insertions in both orientations. The list of chromosomes known to harbor each specific IS element or that commonly acquires each element was obtained from the more detailed catalog of specific IS-promoted mutations, which was compiled by Szybalski (1977). It should be noted that not all of the given recipient chromosomes normally carry an IS element. During IS element insertion and depending upon the specific IS unit, either a 3-4, 5, 9, or 11-12 base pair recipient chromosomal sequence is duplicated in direct order such that one copy occurs at each terminus of the inserted IS unit

Effect of IS element orientation on transcription	Recipient genomes carrying IS units	Length of recipient DNA duplicated at ends of IS elements (b.p.)	Pertinent references
?	*Rhizobium lupini*	?	Puhler and Burhardt (1978)
Polar effects observed for some elements	*E. coli*, *Salmonella*, *S. dysenteriae*, *P. aeruginosa*, *P. mirabilis*, *Streptomyces*; Phage λ, P2; Plasmids R68, ColV, pAMα1	?	Starlinger (1977) Ohtsubo and Ohtsubo (1977) Yagi and Clewell (1977) Schmitt et al. (1979a) Reiss et al. (1978) Szybalski (1977)

At present, there are six named IS elements, IS*1* through IS*5* and IS*10* as shown in Table 3, plus an assortment of unclassified "insertion" mutations that have been identified in *E. coli* and many other bacterial genera, but have not yet been shown to transpose or delete as discrete units (Starlinger and Saedler 1976; see Szybalski 1977). Very recently, a small transposable element, tentatively named "ISR1", has been identified in *Rhizobium* and has been included in this compilation. In addition, I have chosen to include with the IS elements the gamma-delta (γ-δ) sequence which is a normal constituent of the *E. coli* and F plasmid genomes. This segment is now known to transpose *recA*-independently as a discrete unit (Guyer 1978). Although it is somewhat larger than the other IS elements, the γ-δ element appears to behave similarly to them in promoting genetic rearrangements. Unlike the larger transposable elements, it is not yet known to express any phenotypic functions and, thus, bridges the gap between the "simple" IS units and the "more complex" larger transposable elements. The average nucleotide base composition of IS*1* and IS*2* has been determined to be about 50% G+C, comparable to that of the *E. coli* genome (Schmidt et al. 1976; Ghosal et al. 1979b; Ohtsubo and Ohtsubo 1978). Early studies, with λ transducing phage DNA carrying IS*1* or IS*2*, employing DNA-DNA hybridizations, as well as more recent hybridization and electron microscope heteroduplex studies have shown that in *E. coli* K-12 IS*1* exists as about 8 copies per genome (Saedler and Heiss 1973), IS*2* as $\geq$ 7 copies per genome (Saedler and Heiss 1973; Deonier et al. 1979), IS*3* as $\geq$ 5 copies per genome (Deonier et al. 1979), $\gamma\delta$ as $\geq$ 4 copies per genome (M. Guyer, personal communication), and IS*4* and IS*5* as $\geq$ 1 copy each per genome (see Starlinger and Saedler 1976). First discovered in the intensively studied *E. coli* genetic system, one or more classes of the bona fide IS elements have now been found in the related genera *Salmonella*, *Citrobacter*, and *Shigella*, on the phage and plasmids of enteric bacteria, and in the gram-positive bacteria *Bacillus subtilis* (Rak, cited in Starlinger and Saedler 1976; see review by Kleckner 1977; Saedler and Ghosal 1977). Due to the conjugal or infective promiscuity of various plasmids and phage, one would expect a wide dissemination of these and other transposable elements. In fact, IS-like sequences have been detected physically and/or genetically in the diverse genera *Shigella* and *Proteus* (Ohtsubo and Ohtsubo 1977) as well as *Pseudomonas* (Jacob et al. 1977; Reiss et al. 1978), *Streptomyces* (Bibb and Hopwood 1977), *Streptococcus* (Yagi and Clewell 1977), *Staphylococcus* (Novick et al. 1979), and *Rhizobium* (Puhler and Burkardt 1978). However, the sequence relationships among these IS-like elements and the bona fide IS elements have not been established and, to date, only limited data are available on the distribution of known IS elements in bacteria.

Strong polar mutations occur spontaneously in the *E. coli* Lac or Gal operons at a frequency of 10^{-6}-10^{-7}/cell, which is probably a direct reflection of transposition or other recombinational alterations involving any transposable element normally carried by the bacterial chromosome. For this reason, it has been impossible to determine the exact transposition frequency for any specific IS element. Assuming fairly random distribution of IS-

promoted events and knowing that *E. coli* contains about 3000 genes, one can say that the observed transposition frequency has been relatively low ($\sim 10^{-5}$), at best, for most IS units. However, IS element transposition is *recA*- independent and the transposition frequency almost certainly varies with the specific receptor site involved. Examination of spontaneously occuring mutations in *E. coli* and lambda phage has revealed that IS element insertion alone can cause approximately 10-15% of all mutations within a single gene (see review by Starlinger and Saedler 1972, 1976). IS*1* was the most frequently identified insertion mutation. However, it is not known if the small size, high copy number per chromosome, and/or some other attribute of this element is responsible for this phenomenon. Following insertional inactivation of a gene, the function of the mutated gene can be restored at frequencies of 10^{-3}-10^{-8} depending on the IS element, its orientation, and the gene involved (Starlinger and Saedler 1976). Restoration of gene function implies that precise excision of the IS element has occurred and indicates that initial insertion usually occurs without altering the wild-type bacterial gene sequences. The excision of IS elements, known to be *recA*-independent, is probably also promoted by a specialized recombination system. Imprecise excision of an IS unit from a mutant might cause relief of polarity, but continued loss of the mutated gene function. Though such an event has not yet been demonstrated for the IS elements, imprecise excision of phage Mu and larger transposable elements does occur.

The physical aspects of IS elements, which were deduced from DNA sequence analyses, are listed in Table 3 and discussed with the larger, transposable elements in a later section. Also, current theories of IS unit transposition are discussed in the section on mechanisms of transposition.

1) Abnormal chromosomal rearrangements. Insertion and precise excision of IS elements appear to involve enzyme recognition of specific sequences at the IS element termini, though none of the enzymes involved has been isolated. Moreover, specialized recombination events involving these same IS element termini are seemingly responsible for chromosomal deletions and probably for duplication, inversion, transposition, and plasmid integration events. The presence of IS*1* (and perhaps IS*2*) within an operon leads to as much as a 1000-fold increase in deletion formation frequency in this area of the chromosome, yielding deletions in one per every 10^4 cells analyzed. The deletions, which can be from several hundred to as large as 20,000 nucleotide pairs in length, generally terminate at either end of the integrated IS element and extend outward to non-randomly distributed end points within the adjacent bacterial sequences, leaving the original IS element intact (Reif and Saedler 1975, 1977; see Fig. 11). Chromosomal deletions, similar to the deletions commonly observed at the site of Mu phage integration, can also be formed during IS element insertion (see Starlinger and Saedler 1976). Recently, Nevers and Saedler (1978) have identified a function, termed *hfl*, which maps at 61 minutes on the 100 minute *E. coli* genetic map, that is needed for high

DELETION FORMATION

Fig. 11. Deletion formation mediated by transposable elements. The [illegible] represents a portion of a chromosome containing an inserted transposable element, depicted by the [illegible]. Deletion occur unidirectionally from either end of the transposable element outward to non-randomly distributed points within the adjacent chromosomal sequences. The sequences deleted in a hypothetical deletion event are represented by each [illegible]. Following a primary deletion event, as shown here, the transposable element is left intact and can transpose or cause secondary and tertiary deletion events

frequency IS1-mediated deletion formation. Cells lacking this function show a 90-99% decrease in IS1-mediated deletion formation. Additionally, IS1-promoted deletion formation at 42°C vs. 32°C is decreased to different degrees in separate mutants, suggesting that the enzyme-DNA complex, but not the enzyme itself, is temperature dependent (Reif and Saedler 1977). No other requirements of IS-mediated deletions have been identified. It is important to note that precise excision of IS1 in a mutant lacking the *dnaA* function is reduced ~6-fold and may indicate some linkage between precise excision and deletion formation (Nevers and Saedler 1978).

It appears likely that IS elements mediate a variety of chromosomal rearrangements, but confirming data are presently scarce. In addition to the data presented above, IS1 has been reported recently to mediate deletion, transposition, duplication, inversion, and plasmid cointegration events (Iida and Meyer 1979; Shapiro and MacHattie 1979). The participation of IS units as structural components of the more complex transposable elements as well as the involvement of IS elements in bacterial evolution will be discussed later.

c) Regulation of the Expression. Insertion of an IS element, beyond simply abolishing the function of the affected gene, can, depending on its orientation, affect the expression of promotor-distal genes in an operon. The mechanism(s) of polarity is not well understood. However, recent evidence suggests that there are non-random specific sites on DNA, both within and outside of genes, at which RNA polymerase and attached mRNA molecules are released from DNA through the action of a specific protein transcriptional termination factor termed rho (Roberts 1976). Current data suggest that rho initially interacts with nascent mRNA instead of directly with DNA, but that rho can not bind to actively translated mRNA regions. Successful rho attachment to the DNA-bound mRNA somehow signals the RNA polymerase to terminate transcription. Polarity, then, appears to be a composite process. In the first step, translation of the mRNA is terminated at a nonsense codon, created either by substitution or frameshift mutation or carried by an insertion element. Subsequently, rho can attach to the non-translated mRNA molecule at a specific

rho-recognition sequence that is promotor-distal to the nonsense codon, an event which signals the RNA polymerase to terminate transcription. This line of logic is strengthened by the finding that the general polarity suppressor, *suA* (a mutant of the wild-type allele that produces rho protein), can partially suppress the polarity caused by IS*1*, IS*2*, and IS*4* in the *lac* and *gal* operons (Malamy 1970; Malamy et al. 1972; Das et al. 1976; Besemer and Herpers 1977; Sommer, Cullum, and Saedler, personal communication). Since transcription occurs unidirectionally from a promotor sequence, only one strand of an inserted IS element is transcribed along with the genes of an operon. IS*1* and IS*2* are known to exert polar effects when inserted in either orientation within an operon and these elements apparently encode nonsense codons (Ohtsubo and Ohtsubo 1978) in both DNA strands. IS*3* exerts polar effects in orientation I, by original definition, and acts as a transcriptional promotor in orientation II with respect to an operon (Saedler et al. 1974). In addition to encoding a nonsense codon in the polar orientation I, in vitro transcription studies with *λgal* DNA carrying IS*3* in this orientation in the Gal operon indicate that this element also contains a rho-sensitive transcriptional termination site (Decrombrugghe et al. 1973). As discussed below, IS*3* can also act as a genetic promoter, a sequence that binds RNA polymerase and initiates transcription. When the IS*3* element is in orientation I, this element might sometimes initiate transcription in a direction opposite to that of the operon in which it is inserted. It is thought that the RNA polymerase molecules initiated by the IS unit collide with those polymerase molecules initiated on the opposite DNA strand by the operon promoter, causing an additional polar effect on operon expression. Therefore, different IS elements appear to utilize several different mechanisms that result in the overall decrease in expression of promoter-distal genes in an operon (see Starlinger and Saedler 1976).

At least one bona fide IS element, IS*3*, has been found that can positively affect gene expression. IS*3* can behave as a highly efficient genetic promoter when inserted in orientation II as opposed to its polar orientation (I). When inserted in orientation II within the *gal* operon control region, IS*3* has been observed to mediate the expression of more promoter-distal genes at a rate three fold higher than the fully induced wild-type operon (Saedler et al. 1974). Unexpectedly, DNA sequence analysis of one IS*3* element has revealed a rho-sensitive termination site in the strand expressed in orientation II, but no sequence that is similar to known genetic promoters (Ghosal et al. 1979b). Current evidence would suggest that IS*3* in orientation II does not encode a constitutively expressed genetic promoter, but that some internal sequence rearrangement may generate promoter function (Ghosal et al. 1979b; see [illegible] versus [illegible] in Pilacinski et al. 1977). No other bona fide IS elements are known to behave as genetic promoters. Thus, operon expression can be controlled by the insertion of an IS*3* element which depending upon its orientation can either enhance or prevent transcription (Saedler et al. 1974). Albeit the IS*3* element has not yet been shown to invert its orientation while remaining at the same insertion site, the unusual ability of IS*3* to control gene expres-

sion is somewhat analogous to a recently described invertible element. Inversion of an 800 base pair IS-like segment has recently been shown to control the alternate expression of the H1 or H2 flagellar antigens in [illegible] (Silverman et al. 1979; Zieg et al. 1978). In contrast, genetic variation caused by inversion of the G segment of Mu, D108, P1, or P7 viruses, as discussed earlier, appears to be a fundamentally different regulatory phenomenon. Current evidence indicates that a promoter located outside of the G segment is responsible for transcribing essential genes within the G segment. Perhaps when the G segment is positioned in one orientation, the essential G segment genes are expressed and viable phage are produced, and vice versa. Alternatively, two different sets of essential genes controlling host range may be located on different DNA strands of the G segment so that only one set and a specific host range is expressed for each orientation (Howe 1978). Therefore, genetic inversion, which is probably mediated by specialized recombinational processes, results in the regulation of gene expression in at least two different ways and now seems to be a not too uncommon process.

Several independently isolated mutants containing IS[illegible] and IS[illegible] have been used to analyze the DNA sequence of these elements (Calos et al. 1978; Grindley 1978; Ohtsubo and Ohtsubo 1978; Ghosal et al. 1979b; Johnsrud 1979). Although these data are discussed in more detail later, several observations are pertinent here. Certain IS[illegible]-mediated Gal-negative mutants have been found to revert to an unstable, intermediate level constitutive utilization of galactose. Recent analyses of these revertants show that the IS element, though remaining physically in the polar orientation (I) with respect to the operon, now promotes intermediate level transcription. DNA sequence studies show that the revertant IS[illegible] elements, IS[illegible]-[illegible] and IS[illegible]-[illegible], each contain a 54 or 108 base pair complex internal duplication which was probably formed during replication (Ghosal and Saedler 1977, 1978; Ghosal et al. 1979a). Thus, variants of IS[illegible] in orientation I also exhibit genetic promoter activity. Additionally, in separate studies, comparison of the DNA sequences of two different IS[illegible] elements (Johnsrud 1979) or two different IS[illegible] elements (Ghosal et al. 1979b) suggest that small changes in independent isolates of IS elements do occur. Surprisingly then, although IS elements transpose as discrete units, and elements of the same IS class appear grossly homologous by many techniques, even these small DNA segments appear to be constantly evolving.

[illegible]

[illegible]. With the widespread use of antibiotics, a phenomenal increase in the number of bacteria resistant to antibiotics has been observed over the past 20 years. Conjugally transferable antibiotic resistance plasmids (R plasmids) were found to be responsible for this rapid dissemination of resistance (Falkow 1975). Considerable genetic evidence, amassed during the 1960's, indicated that the plasmid DNA segments encoding resistance to one or more medically relevant antibiotics could recombine with

phage, bacterial, or other plasmid chromosomes (see reviews by Cohen and Kopecko 1976; Cohen 1976). Subsequent to the development of techniques to isolate and physically analyze entire plasmid chromosomes, a variety of discrete transposable DNA segments that specify resistance to one or more structurally distinct groups of antibiotics have been identified (Table 4). In the initial description of a large transposable element that encodes β-lactamase production (i.e., penicillin resistance phenotype), Hedges and Jacob (1974) proposed the term *transposon* to define specific DNA sequences with transposition potential. This term, now in common usage, has generally been applied to the larger transposable elements that will be discussed in this section.

b) Molecular Nature and Host Range. Transposons (abbreviated Tn's) can be defined as large DNA segments, which express a phenotypically identifiable trait(s) unrelated to their own insertion, that are capable of *recA*-independent (specialized) transposition, usually in either of two physical orientations, as a discrete non-permuted unit. As shown in Table 4, these elements range in size from 2000 to greater than 80,000 nucleotide base pairs (b.p.). In addition to causing insertional inactivation of a gene, most transposons, like IS elements, also exert polar effects on the more promoter-distal genes in an operon (see Kleckner 1977). Though the initially characterized Tn's all encoded enzymes responsible for antibiotic resistance, more recently Tn's encoding resistance to heavy metal ions (e.g., Hg^{2+}, Stanisich et al. 1977) or enzymes involved in the metabolism of lactose (Cornelis et al. 1978), raffinose (Schmitt et al. 1979b), and toluene, xylene or salicylate (Chakrabarty et al. 1978; Jacoby et al. 1978) have been identified. Furthermore, the genes for enterotoxin production (So et al. 1979) and the genes for synthesis of the K-88 bacterial surface antigen that is responsible for intestinal colonization (Schmitt et al. 1979b), previously identified on plasmids, have now been shown to exist on discrete transposable elements (Table 4). Very recently, the *bio-gal* gene sequences of the *E. coli* chromosome have been observed to transpose at a relatively high frequency (Palchaudhuri et al. 1979; Wolf 1979). Transposable DNA segments have now been identified on the plasmids or host chromosomes of these genera: *Escherichia*, *Citrobacter*, *Salmonella* (Roussel et al. 1979), *Serratia* (Hedges et al. 1977), *Proteus* (Ohtsubo and Ohtsubo 1977), *Shigella* (R100 plasmid, see Ohtsubo and Ohtsubo 1977, Kopecko et al. 1978), *Yersinia* (Cornelis et al. 1978), *Klebsiella* (Berg et al. 1975), *Pseudomonas* (Hedges and Jacob 1974; Chakrabarty et al. 1978; Jacoby et al. 1978), *Vibrio* (Beringer et al. 1978), *Streptococcus* (Tomich et al. 1979), *Staphylococcus* (Falkow et al. 1977), *Bacteroides* (Novick et al. 1979), and *Bacillus* (see Saedler and Ghosal 1977). Also, the fact that conjugally promiscuous replicons like RP4 can transfer to and replicate in many genera not listed above would suggest that most if not all bacteria contain transposable elements.

Physical examination of all transposable elements, including IS units, mainly by electron microscope heteroduplex techniques and also by DNA sequence analyses has revealed a characteristic structure for these discrete elements (see Fig. 12). In all cases thoroughly examined, the IS or Tn unit comprises central DNA

Table 4. Phenotypically-identifiable transposable elements

Tn element designation	Tn-encoded properties	Total Tn length (b.p.)	Length and orientation of internal, terminal repeat sequences (b.p.)	Restriction enzyme susceptibility	
				Cleaved by	Not cleaved by
Tn*1*	Ap^R	∿ 4,600	∿ 40; inverted	*Bam*HI *Hae*II,III *Hin*cII	
Tn*2*	Ap^R	∿ 4,600	∿ 40; inverted	Same as Tn*1*	*Eco*RI
Tn*3*	Ap^R	∿ 4,600	∿ 38; inverted	Same as Tn*1*	*Eco*RI
Tn*401*	Ap^R	∿ 4,600	∿ 40; inverted	Same as Tn*1*?	
Tn*801* Tn*802*	Ap^R	∿ 4,600	∿ 40; inverted	Same as Tn*1* + *Pst*I	
Tn*901*	Ap^R	∿ 4,600	∿ 40; inverted	Same as Tn*1*	*Eco*RI *Sal*I *Hpa*I
Tn*902*	Ap^R	∿ 4,600	∿ 40; inverted	Same as Tn*1*?	
Tn*1701*	Ap^R	∿ 4,600	∿ 40; inverted	Same as Tn*1*	
Tn*4*	Ap^R, Sm^R, Su^R, Hg^{2+R}	∿20,500	< 140; inverted	Same as Tn*1* + ?	
Tn*21*	Sm^R, Su^R	∿15,700	< 140; inverted	*Eco*RI	
Tn(Aβ)	Ap^R, Sm^R	∿14,750			
Tn(?)	Ap^R, Cm^R, Sm/Sp^R, Su^R, Tc^R	∿28,800		*Eco*RI + ?	
Tn*5*	Km^R	∿ 5,200	∿1450; inverted	*Hin*dII,III	
Tn*6*	Km^R	∿ 4,100			

Original chromosomal source of Tn	Length of recipient DNA duplicated at ends of Tn (b.p.)	Transposition frequency (events/cell)	Pertinent references
Pseudomonas plasmid RP4	5	10^{-2}	Hedges and Jacob (1974) Hernalsteens et al. (1977)
Salmonella plasmid RSF1030	5	-	Heffron et al. (1975) Rubens et al. (1976)
Salmonella plasmid R1-19	5	10^{-2}-10^{-5}	Kopecko and Cohen (1975) Kretschmer and Cohen (1977) Ohtsubo et al. (1979) Cohen et al. (1979)
Pseudomonas plasmid RP1	5?		Bennett and Richmond (1976) Grinsted et al. (1978)
Pseudomonas plasmid RP1	5?	10^{-2}-10^{-4}	Benedict et al. (1977)
Salmonella plasmid pRI30	5?	-	Embden et al. (1978)
E. coli phage P7	5?	-	Yun and Vapnek (1977)
Salmonella plasmid NTP1	5?	-	Yamada et al. (1979)
Salmonella plasmid R1-19	-	10^{-6}-10^{-7}	Kopecko and Cohen (1975) Kopecko et al. (1976)
Salmonella plasmid R100-1	-	-	Kopecko et al. (1976) Nisen et al. (1977)
Serratia plasmid R938	-	-	Hedges et al. (1977)
Salmonella ordonez	-	-	Roussel et al. (1979)
Klebsiella plasmid JR67	9	10^{-3}-10^{-2}	Berg et al. (1975), Berg (1977) Davies et al. (1977) Allet (1979)
E. coli plasmid JR72			Berg et al. (1975)

Table 4 (continued)

Tn element designation	Tn-encoded properties	Total Tn length (b.p.)	Length and orientation of internal, terminal repeat sequences (b.p.)	Restriction enzyme susceptibility	
				Cleaved by	Not cleaved by
Tn*601*	Km/Nm^R	~ 3,100	~1000; inverted	*Hin*dIII,III + ?	
Tn*602*	Km/Nm^R	~ 3,100	~1000; inverted	*Bgl*II *Hae*III *Hga*II *Hja*I *Hha*I *Hin*dIII	
Tn*7*	Tp^R,Sm^R	~12,750	< 150; inverted	*Hae*III *Sau*3AI *Hin*dIII	
Tn*71*	Tp^R,Sm^R	~12,750		Same as Tn*7*	
Tn*72*	Tp^R,Sm^R	~12,750		Same as Tn*7*	
Tn*402*	Tp^R	~ 7,500			
Tn*9*	Cm^R	~ 2,500	IS*1*; direct	*Bst*EII *Eco*RI + Same as IS*1*	
Tn*1681*	Heat stable enterotoxin	~ 2,060	IS*1*; inverted	*Hin*cII + Same as IS*1*	
Tn(R-det)	Cm^R,Sm^R,Su^R	~23,000	IS*1*; direct	-	-
Tn*1*	Tc^R	~ 9,300	~1400; inverted (not IS*1*)	*Aha*II *Hae*III *Hga*I,II *Eco*RI *Hin*dIII *Hga*I *Sal*II	*Bst*EII *Bgl*I *Bam*HI *Sal*I *Xho*I *Xma*I

Original chromosomal source of Tn	Length of recipient DNA duplicated at ends of Tn (b.p.)	Transposition frequency (events/cell)	Pertinent references
Salmonella plasmid R6	9?	-	Davies et al. (1977)
Salmonella plasmid R6-5	9	-	Oka et al. (1978) Nomura et al. (1978)
E. coli plasmid R483	-	$5 \cdot 10^{-4}$	Barth et al. (1976) Barth and Datta (1977) Barth (personal communication)
E. coli plasmid R721		-	Barth and Datta (1977)
E. coli plasmid pBW1		-	Barth and Datta (1977)
Klebsiella plasmid R751			Shapiro and Sporn (1977)
Shigella plasmid R100	9	10^{-6}-10^{-7}	Kondo and Mitsuhashi (1964) Gottesman and Rosner (1975) MacHattie and Jackowski (1977)
E. coli plasmid ST	9		So et al. (1979)
Shigella plasmid R100-1	9?		Arber et al. (1979) Hu et al. (1975)
Shigella plasmid R100	9	10^{-6}-10^{-7}	Kleckner (1977, 1979) Kleckner et al. (1975, 1978, 1979a,b) Foster et al. (1975) Kleckner and Ross (1979)

Table 4 (continued)

Tn element designation	Tn-encoded properties	Total Tn length (b.p.)	Length and orientation of internal, terminal repeat sequences (b.p.)	Restriction enzyme susceptibility	
				Cleaved by	Not cleaved by
Tn*1721*	Tc^R	∿10,700	< 38; inverted	*Eco*RI *Sma*I *Hin*dIII *Sac*II *Pst*I *Hpa*I *Sal*I	*Bam*HI *Bgl*II *Xho*I *Kpn*I
Tn*1771*	Tc^R	∿10,800	< 50; inverted		
Tn*551*	Em^R	∿ 5,200	< 100; inverted	*Bgl*II *Hpa*I	
Tn*917*	Em^R	∿ 4,500			
Tn*501*	Hg^{2+R}	∿ 7,800	< 150; inverted	*Eco*RI *Hin*dIII *Sal*I	*Pst*I *Sac*II *Sma*I
Tn*951*	lactose catabolism	∿16,600	∿ 100; inverted	*Bam*HI *Eco*RI *Hin*dIII *Pst*I	
Tn(Tol)	Toluene & Xylene catabolism	∿52,500	?		
Tn(Raf) (Tentative)	Raffinose catabolism, H_2S & K88 antigen	∿40,000 - 60,000	IS*1*; direct		
Tn(Ti) (Tentative)	Tumor induction	∿16,500		*Sma*I *Hpa*I ?	
Tn(Sal)	Salicylate degradation	30,000			
Tn(his-gnd)	Histidine synthesis	44,000	1400; inverted		
Tn(Lac)	Lactose catabolism	80,000	IS*3*; inverted		

Original chromosomal source of Tn	Length of recipient DNA duplicated at ends of Tn (b.p.)	Transposition frequency (events/cell)	Pertinent references
E. coli plasmid pRSD1	5		Schmitt et al. (1979a,b) Mattes et al. (1979)
E. coli plasmid pFS202			Schöffl and Burkardt (1979) Schöffl and Puhler (1979)
Staphylococcus plasmid PI258		10^{-4}-10^{-5}	Novick et al. (1979)
Streptococcus plasmid pAD2			Tomich et al. (1979)
Pseudomonas plasmid pVS1		10^{-1}-10^{-2}	Stanisich et al. (1977) Bennett et al. (1978a)
Yersinia plasmid pGC1		10^{-4}	Cornelis et al. (1978,1979) Cornelis et al. (in preparation)
Pseudomonas Tol plasmid			Chakrabarty et al. (1978) Jacoby et al. (1978)
E. coli plasmid pRSD2			Schmitt et al. (1979b)
Agrobacterium Ti plasmids			Hernalsteens et al. (1977) Schell and van Montagu (1977) Chilton et al. (1978)
Pseudomonas Sal plasmid			Chakrabarty et al. (1978)
E. coli		10^{-2}-10^{-4}	Wolf (1979 and pers. comm.) Palchaudhuri et al. (1979)
E. coli phage P1*Clr*c			Cornelis et al. (in preparation)

Tn numbers are those assigned by E.M. Lederberg, Plasmid Reference Center, Dept. of Medical Microbiology, Stanford University. Those Tn designations given in parentheses describe certain transposable elements for which numbers have not yet been given. The Tn-encoded properties include resistance to ampicillin (Ap^R), chloramphenicol (Cm^R), erythromycin (Em^R), kanamycin (Km^R), neomycin (Nm^R), spectinomycin (Sp^R), streptomycin (Sm^R), sulfonamide (Su^R), tetracycline (Tc^R), trimethoprim (Tp^R), and divalent mercury (Hg^{2+R}). Also, production of heat stable enterotoxin, K88 antigen, and H_2S, plus degradation of lactose, raffinose, toluene, xylene and salicylate are Tn-encoded properties. Recently, a segment of the *E. coli* chromosome, which encodes the *his-gnd* loci (genes for the biosynthesis of histidine and synthesis of gluconate dehydrogenase, respectively), has been shown to transpose to plasmids and phages. Also, recent evidence suggests that the tumor-inducing segment of Ti plasmids transposes to plant cells as a specific unit, but this has not been proven.

Total Tn size, as well as the length and orientation of the internal repeat sequences at Tn termini are given in nucleotide base pairs (b.p.). Tn*1*, *2*, *3*, *401*, *501*, *551*, *903*, *917*, and *1681* appear to be homologous Ap^R elements, now collectively termed TnA. Tn*3* is a composite transposon apparently consisting of Tn*1* inserted into Tn*21* (Kopecko et al. 1976). Tn*501* and *551* are probably identical elements. Tn*5*, *10*, and *11* also appear to be homologs obtained from different plasmids. Tn 1721 and 1771 appear to be identical Tc^R Tn's. Tn*4*, Tn(R-det), Tn(Raf), and Tn*1681* have direct or inverted repeats of IS*1* at their termini. The inverted sequences at the termini of Tn*21* are not IS*1*, *2* or *3*, contrary to many published reports (see Kleckner and Ross 1979). A composite of published restriction endonuclease cleavage susceptibilities of various Tn's is given. However, one should be aware that there is noticeable variability in restriction patterns of similar Tn elements (Yamada et al. 1979). Since most Tn's have been isolated from plasmids, the bacterial host and/or plasmid in which the Tn was originally detected are listed. Also, the length of the recipient DNA sequence that is duplicated at the ends of a Tn element is given in b.p. A compilation of reported transposition frequencies, listed as transposition events per cell, is given, usually as a range. In most Tn identifications, transposition frequency was not assessed. Certain listed Tn properties are based on assumption (i.e., likeness to characterized Tn's) and those have been denoted by an accompanying question mark. See Campbell et al. (1979) for transposon nomenclature

sequences bracketed by either a directly or an inversely repeated terminal DNA sequence. For example, IS*1* comprises a 1250 b.p. central segment containing a 40 b.p. sequence that is repeated, imperfectly, in inverse fashion at each terminus (Table 3). Tn*3* consists of approximately a 4500 b.p. segment flanked by inverted repeats of a 38 b.p. sequence (Table 4). Though most transposable elements are structurally defined by inverted repeat DNA sequences, the Tn*9* (Cm^R), Tn(Raf), and Tn(R-det) contain direct repeats of the IS*1* sequence at their termini. However, since the ends of IS*1* contain a small inverted repeat sequence, these Tn's are actually bracketed by small inverted repeats at their termini. Thus, all IS's and Tn's for which the ends have been characterized to date, contain an inverted terminal repeat sequence. The total length of each transposable element as well as the length and orientation of the internal repeated terminal sequences, if known, are given in Tables 3 and 4 for both IS and Tn units. Examination of

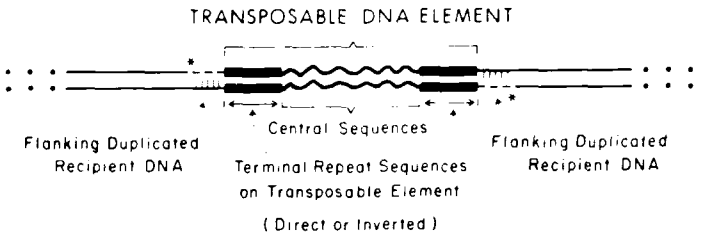

Fig. 12. The structure of transposable elements. This unscaled diagram illustrates a transposable DNA element, which is delineated by the [illegible] and [illegible], inserted within a recipient molecular DNA sequence, which is depicted by the [illegible]. Both IS and Tn units are composed of a central DNA segment [illegible] flanked by terminal sequences [illegible] that are repeated either in direct or inverted order. Apparently during insertion, staggered single-strand cleavage occurs at the sites marked by [illegible], and the extended single-strand ends of the recipient molecule are joined to the transposable element. Subsequent gap filling DNA synthesis [illegible] and ligation creates duplicated recipient molecular sequences at the ends of the inserted Tn or IS element

the junctions of IS or Tn elements with recipient molecular DNA has revealed the existence of directly repeated recipient molecular sequences of 3 to 4, 5, 9, or 11 to 12 b.p. at each end of the inserted element (Fig. 12). Though the nucleotide composition of the repeated recipient sequence varies from insertion site to insertion site for a particular element, each transposable element is always associated with a repeated recipient DNA sequence of specific length, as shown in Tables 3 and 4 (Calos et al. 1978; Grindley 1978; Johnsrud 1979; Ghosal et al. 1979b; Tu and Cohen 1980). Apparently, during Tn or IS insertion an enzymes(s) creates a single-strand cleavage, staggered by 3 to 4, 5, 9, or 11 to 12 b.p., in each recipient DNA strand and the extended single-strand ends are joined to the transposable element, with the complementary strand at each end being newly synthesized (see Fig. 12; also see section on transposition mechanisms).

The large inverted repeat termini located on many transposons make these elements easily identifiable in the electron microscope. As illustrated in Fig. 13, after denaturation and intrastrand annealing of a DNA segment that carries a Tn which contains inversely repeated termini, one sees characteristic hairpin-loop or stem-loop structures in which the double-stranded stem represents the reannealed, inversely repeated Tn termini. In addition to forming characteristic stem-loop structures of constant size, insertions of any transposon will increase the size of the recipient molecule by a discrete length. Further evidence for a specific transposition event can be obtained by extensive restriction endonuclease analysis of several molecular isolates containing the same putative Tn element, since Tn units transpose as non-permuted DNA segments and consequently retain the same restriction sites.

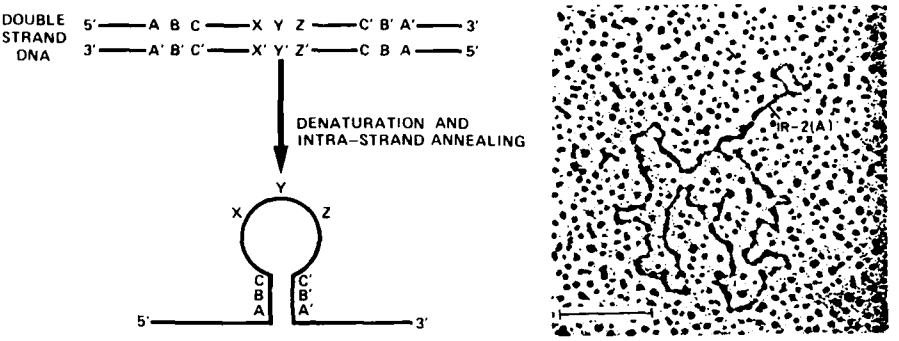

Fig. 13. Characteristic intra-strand structure of transposons with inversely repeated termini. [illegible]: A double-stranded DNA segment containing a transposon with complementary termini. Following denaturation and intra-strand annealing, hairpin-loop or stem-loop structures are observed. [illegible]: Single-strand molecule containing a double-stranded stalk or stem, comprising the Tn termini. The central sequences of the Tn unit (XYZ) are enclosed in the single-strand loop at the top of the stem. [illegible]: Electron micrograph of a self-annealed single-strand of a small plasmid containing the Tn[illegible] element. The double-strand stem represents the 1000 base pair inverted repeat sequences at the termini of this element

Any inserted element can be directly visualized in the electron microscope following heteroduplex formation, a technique in which single-strands of a parental molecule are allowed to reanneal with complementary strands of an identical derivative molecule that contains the inserted element (see Fig. 14). The interaction of two linear DNA single-strands to form a double-stranded segment requires axial rotation of one strand around the other in order to form the DNA helix. Because of structural constraints then, two entirely complementary, but colvalently sealed circular single-strands can not form a complete duplex molecule, but rather end up forming a molecule consisting of a mixture of duplex and single-strand regions. This is exactly what occurs when two entirely complementary DNA strands, in each of which the inverted repeat termini of a transposon have already intra-strand annealed (as shown in Fig. 13b), attempt to reanneal with each other. The sequences in the circular loop region of each strand are structurally constrained (i.e., can not undergo axial rotation) and can only form a partially duplex structure (termed underwound loop). The observation of underwound loops can be diagnostic of short inverted duplications on Tn elements as well as of the presence of new transposable elements (see Broker et al. 1977b).

[illegible]. Transposition of Tn's occurs at frequencies, which range from 10^{-7}-10^{-1} events/cell, that probably depend upon: (1) the bacterial host (e.g., [illegible] strain AB1157 and its derivatives decrease the normal Tn transposition frequency observed in other [illegible] K-12 strains; Hedges et al. 1977); (2) the recipient chromosomal sites (Kleckner

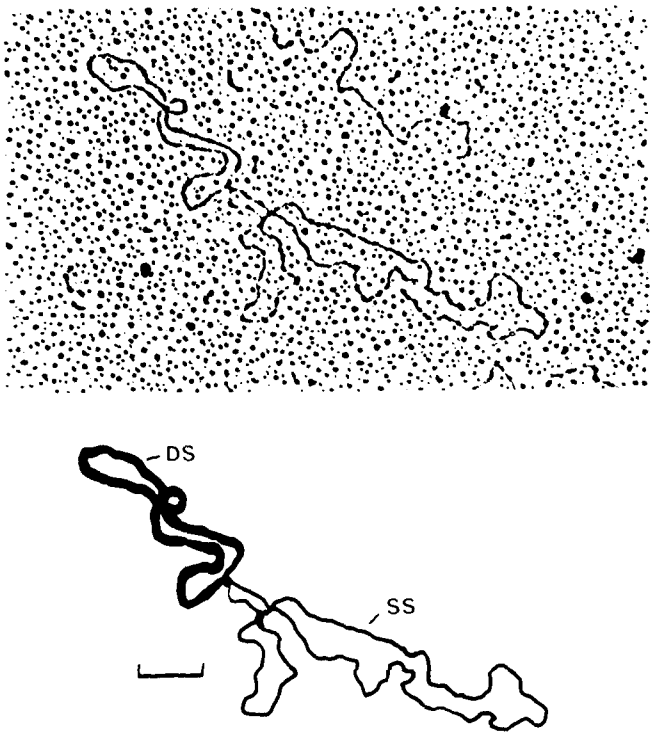

Fig. 14. DNA heteroduplex molecule. Single-strand DNA appears as a thin, uneven line in the electron micrograph, compared to the thicker double-stranded portion of the molecule. In this example, the 9,200 base pair (9.2 kilobase pair, kb) pSC101 plasmid has reannealed with the pSC120 plasmid. pSC120 is a recombinant plasmid made by inserting the 20.5 kb Tn*1* element into pSC101. The tracing below shows a double-stranded (DS) circle that represents all of the pSC101 sequences, with one large single-strand (SS) insertion loop which represents the Tn*1* element. Although not labeled, two small inverted repeat sequences are shown. One occurs at the termini of Tn*1* and the other represents the ends of the Tn*1* element that is located in this composite transposon (for more details see Kopecko et al. 1976). The [illegible] represents 0.25 micrometer

et al. 1979b; Tu and Cohen 1980); and (3) the Tn element itself (see Table 4). Under what appear to be fairly optimal conditions Tn*1*, Tn*3*, or the TnA elements have been observed to transpose at frequencies of one transposition event per every 10 to 1000 cells (Bennett et al. 1977a; Davies et al. 1977; Grinsted et al. 1978). In contrast, other elements like Tn*5* transpose less frequently (10^{-7}-10^{-6}) events/cell; Foster 1977; Kleckner 1977). Insertion in either orientation occurs with apparently equal frequency for all Tn's (Rubens et al. 1976; Kleckner 1978; Cornelis et al. 1979), with the exception of Tn*7* which has the same orientation in 36 separate insertions within RP4 (Barth and Grinter 1977).

Transposition of discrete, non-permuted units implies that specific sequences at the ends of Tn elements are enzymatically recognized during insertion. Recent DNA sequence analyses of various inserted Tn's or IS units shows that for each element the same terminal nucleotide at each end of the element always forms the junction with recipient DNA (Calos et al. 1978; Grindley 1978; Ohtsubo and Ohtsubo 1978; Kleckner 1979; Tu and Cohen 1980). Available information on the recipient chromosomal recognition sites, however, is not easy to interpret. The large distribution of possible insertion sites that have been observed in the *E. coli* chromosome for the representative Tn*A* or Tn*3* elements would suggest that there is little specificity involved at the site of insertion. Like phage Mu, Tn*A* or Tn*3* insertions cause new nutritional requirements in 1-2% of the bacterial chromosomes into which these elements transpose. On the surface, these data would suggest that there is a short, three to five bp recognition sequence that is distributed randomly and often throughout most recipient chromosomes. However, despite the capability of these and other transposons to insert into many different loci, the preponderance of transposon insertions has been found to occur at preferred areas of the recipient chromosomes (termed "regional" or "local" specificity). For example, Kleckner et al. (1979b) recently found that out of 131 independently isolated Tn*10* insertions within the 10,000 bp *S. typhimurium* histidine (*his*) operon, 50 insertions occurred within a single 30 bp region of the *hisG* gene. Foster (1977) previously reported that 18 of 21 independently isolated Tn*1* insertions within the 3520 bp *E. coli lac* gene occurred in a single, small 175 bp region. These are examples of extremely "localized" insertion specificity in which Tn insertion occurs at non-identical, nearby sites and sometimes precisely at the same locus (Kleckner 1979). It is very interesting to note that Johnsrud et al. (1978) have found that 29 of 50 Tn*A* insertions within the *E. coli lac* gene map in the same "recombinational hotspot" that Foster observed for Tn*1* insertions in *lac*, suggesting that, at least, these different Tn's respond to the same recipient recognition sequence.

Very early reports on TnA insertion specificity (Kopecko et al. 1976; Rubens et al. 1976) as well as more recent findings for TnA, Tn , and Tn elements (Barth and Grinter 1977; Kretschmer and Cohen 1977; Grinsted et al. 1978; Tu and Cohen 1980) indicate that these Tn's do not insert at random, but that a large proportion of insertions occur in not-so-compact, recipient genomic regions of 500-1000 bp in length (i.e., "regional" specificity). If a frequently occurring recipient genome recognition sequence for Tn insertion was present, one would not expect clustered insertion sites at nearby loci, but randomly distributed insertions, with separate transposition events occasionally occurring at the same recognition site. Therefore, the non-random, "localized" or "regional" specificity of Tn insertions would argue against a randomly distributed recipient, 3-5 bp recognition sequence. The data of Tu and Cohen (1980) suggest that the "regional" specificity for Tn*A* insertion is due to recipient DNA A+T richness plus homology with the ends of Tn*A*. However, Grinsted et al. (1978) have convincingly established that a

500 bp DNA segment while present in one plasmid behaves as a "recombinationally hyperactive" recipient site for transposition, but the same DNA segment when located adjacent to different sequences in a derivative plasmid does not. Thus, a short, recipient recognition sequence is not sufficient, in itself, for transposition. Furthermore, DNA sequence analyses of various Tn- or IS-recipient DNA recombinational junctions have not revealed any such common recipient DNA recognition sequence (Grindley 1978; Johnsrud et al. 1978; Oka et al. 1978; Ghosal et al. 1979b; Kleckner 1979). Rather, it appears likely that a more complex recipient chromosomal recognition sequence is involved and that the point of cleavage of recipient DNA (i.e., the Tn insertion site) occurs some distance (up to 500 or 1000 bp) from the recognition site. Type I restriction endonucleases, which exhibit this pattern of behavior, or some similar enzyme may be responsible for the observed preferred areas of Tn insertion, as discussed in the section on mechanisms of transposition.

Little is factually known about the mechanism of Tn or IS element transposition except that it occurs independently of homologous recombination systems. Considerable genetic evidence indicates that transposition to a new site does not cause loss of the element at the original locus (Bennett et al. 1977a; Shapiro 1979). As a matter of fact, transposition of any Tn unit is detected 10^2-10^5 times more often than loss of the Tn element by precise excision. This evidence would imply that an obligatory and integrally linked replication/transposition event, like that discussed for Mu phage, occurs with transposable elements. Only a single-strand template or, perhaps, a newly replicated duplex copy of the transposable element would be inserted into the recipient site. Although the energy, enzyme, and structural requirements of transposition are unknown, for the most part, limited data obtained with Tn[illegible] would suggest that in [illegible] transposition efficiency at 37°C is only 10% of that observed at 32°C and transposition does not occur at 45°C (Kretschmer and Cohen 1977). The requirements for DNA, RNA, and/or protein synthesis in transposition have not been established nor have the effects of temperature on the transposition of other transposons. As mentioned previously, the [illegible] and [illegible] genes, which probably encode proteins that are common to several different DNA metabolic processes, affect the frequencies of transposition and excision of transposable elements, but the nature of these affects is not understood (Miller and Friedman 1977; H. Miller, personal communication). Some mechanism apparently exists that controls the frequency of transposition since this frequency appears to reach saturating levels after a period of time. For example, to date the transposition frequency is always 10^2-10^5 higher for Tn[illegible] than Tn[illegible] (Kleckner 1977; Grinsted et al. 1978). In addition, Bennett et al. (1977b, 1978b) have observed that the presence on a recipient plasmid of Tn[illegible] or a TnA derivative that is mutated to β-lactamase non-production ([illegible]) decreases the transposition frequency of a second TnA element to that plasmid, but not to other plasmids in the same cell. Therefore, the presence of a Tn element may, in some cases, suppress subsequent transposition to the carrier plasmid (i.e., it exerts

a *cis*-acting suppressive effect). On the other hand, it should be noted that plasmids carrying two TnA elements have been physically identified, a fact which obsures the importance of the *cis*-acting suppressive effect (Bennett et al. 1978; Holmans et al. 1978).

Considerable effort has been applied to isolating mutants of transposons in order to see if any Tn sites or functions are necessary for transposition. To recap the conclusions before presenting the data, it appears that the inversely repeated sequences at the Tn termini are necessary for enzymatic recognition during transposition. Furthermore, at least Tn*3* and Tn*1* encode a protein(s) that is involved in their respective transposition. Heffron, Falkow, and co-workers have used a variety of novel techniques to generate addition/deletion mutations within the Tn*3* element (Heffron et al. 1977, 1978; Gill et al. 1978). By complementation of the transposition-deficient mutated Tn*3* elements with a *bla*$^-$ Tn*3* (i.e., phenotypically ampicillin sensitive), three classes of defective Tn*3* elements have been detected. Non-complementable mutants that contain a deletion of one terminal inverted repeat sequence demonstrate that the terminal sequences are a structural requirement for transposition. Similar conclusions were obtained by studying deletions of Tn*5* (Davies et al. 1977). Secondly, mutants obtained in approximately one-half of the Tn element could be complemented to transpose at 20% of the normal frequency. These mutants define a *trans*-acting function (RNA or protein) that is necessary for transposition. The third class of mutants occurs in a region surrounding the single *Bam* HI endonuclease cleavage site on Tn*3* and affects both the frequency and type of transposition event. When small insertions are biochemically spliced into this latter region, the transposition frequency is increased tenfold, compared to wild-type Tn*3*, and about 30% of these transposition events are abnormal, i.e., cause the insertion of the entire donor plasmid into the recipient replicon, as shown in Fig. 15c (Heffron et al. 1978; Heffron, personal communication). In the presence of a wild-type Tn*3* that is *bla*$^-$, these latter insertion mutants of Tn*3* transpose as a discrete transposon greater than 99.9% of the time. It appears that the wild-type Tn*3* makes some *trans*-acting function that changes the transposition event back to normal Tn unit transposition (see Fig. 15d). In contrast, Tn*3* mutants that contain deletions of this region which affects the quality and quantity of transposition events are not observed to transpose unless complemented. Complementation of these mutants by a *bla*$^-$ Tn*3* restores the transposition frequency to about 20% of normal levels, but all of these Tn*3* deletion mutants form cointegrate structures upon transpositon, i.e., the entire donor plasmid, flanked by direct repeats of the mutant Tn*3* element, is inserted into the recipient replicon (Gill et al. 1978; see Fig. 15c). The basic interpretations of these data are illustrated in Fig. 15. In addition to the specific terminal sequences needed for transposition, Tn*3* encodes the production of two *trans*-acting proteins (a recently identified 110,000 mol. wt. protein (transposase or recombinase?) and a 19,000-20,000 mol. wt. "regulator" protein; Chou et al. 1979; Dougan et al. 1979; S. Cohen, F. Heffron, personal communication)

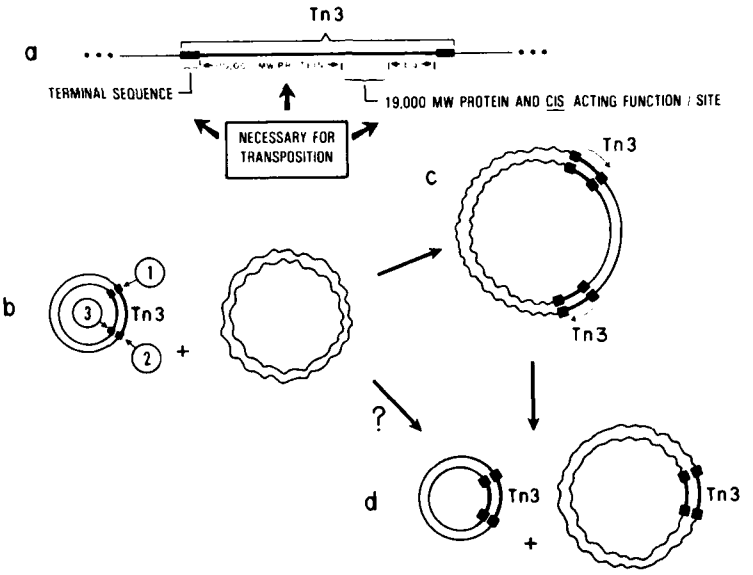

Fig. 15. Molecular and genetic aspects of Tn transposition. (a) Diagramatic depiction of the Tn*3* transposon showing the terminal repeat sequences essential for transposition. In addition, genetic and biochemical studies have revealed the Tn sequences coding for β-lactamase (*bla*) and functions/sites needed for transposition, as shown. The 110,000 mol. wt. protein may be a recombination enzyme, and the 19,000 mo. wt. protein and adjacent *cis*-acting function/site appear to regulate the frequency and type of transposition event as shown below (Gill et al. 1978; Heffron et al. 1978; Heffron, personal communucation). (b) Small donor plasmid carrying the Tn*3* transposon and a larger recipient chromosome, represented by a *circular*, *unspecified* line. (c) The results of studies with mutant transposons suggest that transposition occurs by cleaving the donor molecule at points labeled 1 and 2 in (b) followed by insertion of the entire donor plasmid flanked by direct repeats of the transposon into the recipient molecule. (d) Almost simultaneous processing is thought to occur generating independent donor and recipient replicons each containing one copy of the Tn unit. The regulator protein and *cis*-acting function/site, shown above, appear to be necessary for this normal processing. In the absence of either regulator function or site, the transposition event stops at the intermediate cointegrate stage (also see Meyer et al. 1979; Shapiro 1979). For ease of illustration, the recipient DNA sequences known to be directly repeated at the insertion site have not been drawn.

Current evidence does not eliminate the possibility that normal transposition occurs by initial cleavage of the Tn unit at only one end (labeled 2 in step b), followed by single-strand Tn transfer to the recipient molecule, simultaneous complementary strand synthesis on one or both molecules, cleavage of the other end of the Tn unit (at point 1 in step b), repair synthesis and ligation (see Grindley and Sherratt 1979) to give the molecule depicted in (d). The cointegrate structure (c) may be an aberrant recombinational product caused by lack of the "regulator" function/site and/or some other property (see the text)

and a cis-acting function/site located near the gene for "regulator" protein (see Fig. 15a). Cohen and coworkers have recently demonstrated that the 20,000 mol. wt. regulator protein serves as a repressor molecule that effectively controls a bidirectional genetic transcription unit which includes the 110,000 mol. wt. transposase and production of the repressor protein itself (Chow et al. 1979; Chow, Lemaux, Casadaban and Cohen, submitted for publication). Usually, transposition involves the transient formation of cointegrate structures initially proposed by Kopecko and Cohen (1975) and shown in Fig. 15, which are processed into donor and recipient replicons, each carrying one copy of the transposable element (Fig. 15d). The Tn*A* mutants deleted for the "regulator" protein are also missing the cis-acting function/site, both of which are thought to direct the processing of the cointegrate structure (Gill et al. 1978). Even during complementation, all transposition events involving these Tn*A* deletion mutants remain abnormal because the cis-acting function/site is missing (Arthur and Sherratt 1979). These interpretations are supported by the results of similar studies with mutants of the Tn*3* element, which also suggest that a cis-acting function necessary for processing the normally transient cointegrate, recombinational intermediate is encoded within the Tn sequences (Meyer et al. 1979).

Based on our limited genetic knowledge of transposition, the above interpretations appear quite reasonable. However, other explanations are also plausible. For example, the normal transposition process may not involve the formation of a transient cointegrate intermediate structure (Fig. 15c), but instead may mediate the transfer of a single-strand of the transposon to a new site via the model of Grindley and Sherratt (1979) which is discussed in a later section and in the legend to Fig. 15. The regulator protein and cis-acting function/site might normally limit initial enzymatic cleavage to one end of the element, as described in the legend to Fig. 15. A defective regulator protein and/or lack of the cis-acting function/site might result in loss of control of the enzymatic cleavage and, consequently, the aberrant formation of a cointegrate structure. Cleavage of the transposon at each end but on opposite strands, and insertion into a recipient site which could occur by the model of Shapiro (1979; discussed in a later section), would result in the formation of an aberrant cointegrate structure. In the past year there were several other reports of transposon-mediated plasmid cointegration as depicted in Fig. 15c. When harbored on a multicopy colE_1 plasmid derivative, either Tn*3* or the enterotoxin transposon usually causes, during transposition, the cointegration of the entire donor plasmid bracketed by direct repeats of the Tn element (see So et al. 1979). However, these transposons have not been mutated and usually transpose from a larger plasmid or phage as a discrete element. One wonders if transposon-mediated, plasmid cointegrate formation is enhanced in these small multicopy plasmids because of some inherent property such as their rapid replication. More information on transposition of wild-type and mutant Tn's from large and small plasmids is obviously needed. Additionally, conditional mutants of Tn's and complementation studies among dif-

ferent Tn's might reveal new insight into the transposition process.

To summarize briefly the above information, genetic and physical studies indicate that Tn elements normally transpose as discrete units. It is generally assumed that the very termini of all transposons are essential structures for transposition, but their necessary, minimal length is not known. Thus far, only Tn*A* and Tn*5* have been shown to encode *trans*-acting functions essential to transposition. The transposition process may involve the initial formation of a cointegrate structure followed normally by its resolution into donor and recipient molecules, each carrying one copy of the Tn element.

Reversion of Tn-induced mutations, which is generally associated with loss of the Tn unit, is assumed to be due to precise excision of the Tn element and occurs for all transposons at frequencies ranging from 10^{-9}-10^{-6} (Kleckner et al. 1975; Berg 1977; Foster 1977). Since Tn transposition does not result in loss of the Tn unit at the original site and vice versa (Bennett et al. 1977a; Kleckner 1977), it is probable that Tn excision and transposition occur by different processes (discussed in the section on transposition mechanisms). In addition to undergoing transposition and precise excision events, most Tn's can mediate the rearrangement of nearby chromosomal sequences as discussed below.

d) Aberrant Chromosomal Rearrangements. While searching for reversion of Tn-induced mutations, imprecise excision events were detected at a frequency of 10^{-6}-10^{-4} events per cell by either polarity relief in the *lac* operon (Berg 1977; Foster 1977) or loss of Tn-encoded antibiotic resistance (Kleckner et al. 1979a; Ross et al. 1979b). Imprecise excision generally removes some of the Tn unit including drug resistance (e.g., tetracycline resistance) and, depending upon the Tn element, occurs 10-1000 times more frequently than precise excision (Kleckner 1977). Though known to occur independently of general recombination like precise excision, the mechanisms responsible for imprecise excision are not entirely understood. Physical examination of 26 imprecisely excised Tn*10* elements has revealed several different types of imprecise excision events (Ross et al. 1979a,b). Restriction enzyme analyses revealed that in ~50% of these isolates, only 50-100 bp of Tn *10* sequences remain. DNA sequence analysis of two of these Tn*10* elements has shown that exactly 50 bp of Tn*10* remain in each case. Moreover, the deletion event appears to have occurred between short A+T rich regions internal to and directly repeated at each Tn terminus. Another group of imprecisely excised Tn*10* elements (8 of the original 26) was observed to contain simple internal Tn*10* deletions that uniquely had one deletion end point in very close proximity to either end of the 1400 bp repeat at either Tn*10* terminus. A third group of imprecisely excised Tn*10* elements (6 of 26) contain deletions of all of the Tn*10* sequences internal to the 1400 bp inverted terminal sequences plus concomitant inversion of adjacent sequences. As depicted in Fig. 16, Ross et al. (1979a) have proposed that the internal ends of the 1400 bp Tn termini recombine with some adjacent site, always resulting in concomitant deletion and inversion events.

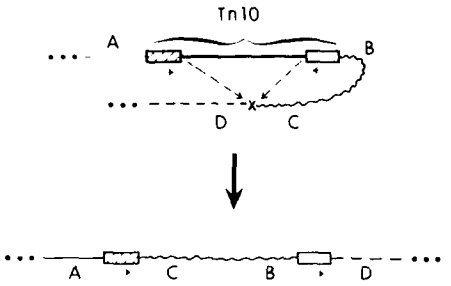

Fig. 16. Transposon-promoted inversions during imprecise Tn unit excision. The Tn*10* transposon is shown here inserted within a chromosome which is represented by *solid, squiggled,* and *broken lines*. Chromosomal regions *A* through *D* are so labeled. The 1400 bp repeats on Tn*10* are shown as *rectangles* and their orientation is indicated by the *arrows* below each. The inside end of each 1400 bp repeat is proposed to recombine (see *dashed arrows*) with some adjacent sequence, denoted here by an *X*. This recombination event results in the loss of the Tn*10* sequences normally contained within the terminal 1400 bp repeat sequences, as well as the inversion of one 1400 bp repeat and some adjacent chromosomal material (for details see Ross et al. 1979a)

Tn*3* has been found to generate, *recA*-independently, deletions which apparently occur from either terminus of the inserted transposon and extend outward to non-random points within adjacent chromosomal sequences, but always leave the Tn element functionally intact (Nisen et al. 1977). Similar results have been reported for Tn*10* (Noel and Ames 1978). Furthermore, in both studies recipient chromosomal regions that appeared as "recombinationally hyperactive" sites for Tn insertion also were found to act as preferred deletion end points, suggesting that transposition and deletion processes are similar.

Tn*9*, Tn*3* and the enterotoxin transposon have been observed under certain conditions to mediate the cointegration of two circular genomes, as mentioned in the previous section. Also, transposon-mediated chromosomal integration of phage λ has been detected (Davies et al. 1977; MacHattie and Shapiro 1978). In addition, Tn*10* has been used experimentally to generate duplications of chromosomal regions as well as to fuse unrelated chromosomal sequences (Kleckner et al. 1977). Though only limited data are presently available, it appears likely that most transposons will be found to generate all of the chromosomal rearrangements that are promoted by phage Mu, as discussed before.

4. Mechanisms of Transposition - Current Theories

DNA sequence analyses of many of the recipient chromosomal sites into which Mu phage, IS elements, or transposons insert seem to bear no great similarity to one another (Allet 1979; Ghosal et al. 1979b). This fact separates, on a mechanistic level, these specialized recombination systems from those of temperate bacteriophages, like λ, which appear to promote phage insertion into only one or a few short, but highly homologous regions of a chromosome. Therefore, though similar in the ability to promote chromosomal rearrangements in the absence of general recombination and without extended sequence homology, specialized recombination systems do display differences. The similarities among transposition events of IS- or Tn-units and Mu phage suggest that these different elements transpose by fundamentally related processes, as detailed below. However, the existence of several different specialized transposition processes which result in the same end product cannot be ruled out at the present time.

a) Essential Features of Specialized Transposition. Genetic and molecular studies of the specific transposition of Mu phage and transposable elements (Tn, IS units) have revealed several general properties among these events. Transposition of an element from one chromosomal site to another does not appear to result in loss of the element at the original locus (Bennett et al. 1977a; Bukhari 1977; Shapiro 1979), a fact that ensures the involvement of replication in this event. Furthermore, insertion of a transposable element or Mu phage into a chromosome results in the direct duplication of 3-4, 5, 9, or 11-12 bp of recipient DNA at the insertion site (see Calos et al. 1978; Grindley 1978; Allet 1979; Ghosal et al. 1979b). The recipient DNA repeat sequences bracketing any specific element (e.g., IS*1*) are always the same length but usually vary in nucleotide composition. Data obtained with a derivative Tn*3*, Tn*9*, or Tn*10* element, experimentally constructed so as to contain non-identical DNA flanking each Tn terminus, indicate that this repeated DNA is not essential for the transposition event (Johnsrud et al. 1978; Kleckner 1979). However, most transposable elements are inserted in one of two possible orientations at virtually any chromosomal site (Bukhari 1977; Kleckner 1977). Thus, the recombination process must involve recognition of the specific Tn or Mu termini. Although independent insertions of some Tn elements have been found at precisely the same locus (Kleckner 1979; Tu and Cohen 1980), most Tn insertions occur in preferred chromosomal regions, whereas Mu phage insertions appear entirely non-specific with respect to the insertion site (Kleckner et al. 1979b; Ljungquist et al. 1979). Based on the above observations of the structural consequences of specialized transposition, several models have been proposed to explain the transposition process.

b) Single-strand Transfer Model. Ljungquist and Bukhari (1977) have provided evidence which suggests that Mu transposition follows or occurs concomitant with Mu specific replication (Bukhari 1977). Additionally, data obtained from transposition studies of TnA elements suggested to Bennett et al. (1977a) that transposition might involve single-strand transfer of the element

and complementary strand synthesis in the donor and recipient molecules. Grindley and Sherratt (1979) have recently described a model for single-strand transfer of any transposable unit. This simple model accomodates transposition without consequent deletion of the donor transposable element and allows for duplication of recipient sequences at the insertion site, as summarized in Fig. 17. Accordingly, one enzymatic activity is responsible for making staggered cuts in the recipient DNA (Fig. 17a). The observations that all transposable DNA units have 3-4, 5, 9, or 11-12 bp repeats of recipient DNA at insertion sites suggest that four separate, probably host-determined, factors provide this target site endonucleolytic nicking activity. A second component of this proposed reaction is an activity that recognizes, specifically cleaves, and transfers one end of a single-strand of the transposable unit to the appropriate site on the nicked recipient DNA (Fig. 17b). This second enzymatic activity may be specified by each transposable unit, as the evidence indicates for Mu, Tn*3*, and Tn*5* (see Grindley and Sherratt 1979). Each transposable unit-specific enzyme apparently interacts with only one of the four common target site nicking proteins. Following ligation of one strand of the transposable unit to a 5' end of the recipient DNA (Fig. 17b), replication proceeds in the recipient molecule by copying the displaced transposed segment. Complementary strand synthesis on the donor molecule is not proposed by this model, presumably because no available primer exists. When complementary strand synthesis is completed in the recipient molecule, the end of the newly synthesized transposable element is ligated to the free 5' end of the recipient (Fig. 17c). Subsequently, the displaced donor strand is retransferred back to the donor molecule and complementary strand synthesis occurs on the recipient strand from the remaining free 3'-OH end that was initially created by the target site nicking activity (Fig. 17d). The result is that both strands of the transposable unit are conserved in the donor molecule and a newly replicated transposable element exists in the recipient. Interruptions in the proposed process could generate partial or complete semi-conservatively replicated transposable units. Also, an aborted transposition attempt via this model to a site adjacent to the original transposable unit could generate deletions with one end point at the transposable unit (see Grindley and Sherratt 1979). By this proposal, specialized transposition is a separate process from precise excision which must result in the double-stranded removal of one flanking recipient repeat sequence in addition to the entire transposable segment. Precise excision may involve *RecA*-independent recombination between the short recipient DNA repeat sequences that flank transposed elements (see section on *recA* -independent systems for recombination). A less likely and yet untested alternative is that transposition is a non-reciprocal exchange that usually results in loss of the donor molecule (Bennett et al. 1977a; Bukhari 1977).

c) The Fusion Model for Transposition. Over the past few years there were repeated observations in which transposable units caused the fusion of two replicons, where only one of the replicons initially contained the element (Faelen et al. 1975; Heffron et al. 1973; Shapiro 1979). Although the model presented above can be adapted to creating replicon fusions (see Grindley and

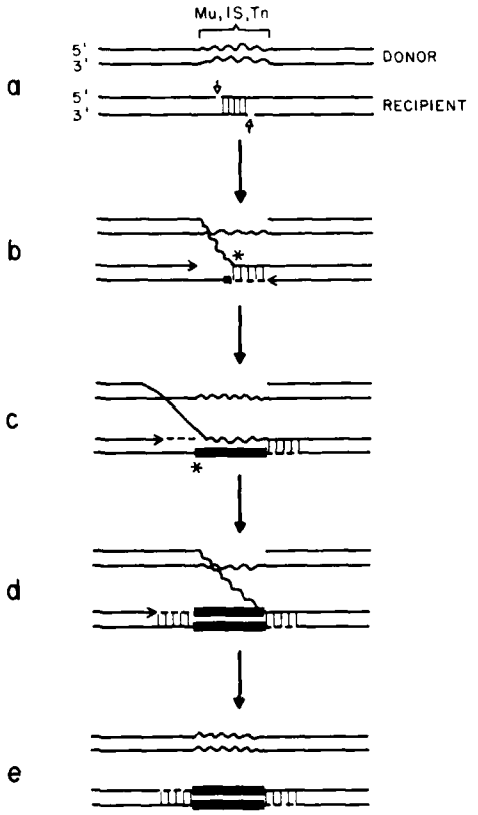

Fig. 17. A single-strand transfer model for transposition. Donor and recipient double-stranded DNA regions are represented by *pairs of horizontal lines*. The discrete transposable DNA unit is depicted by *squiggled lines*. Newly replicated chromosomal DNA is represented by *dashed lines* while newly synthesized transposable segment sequences are depicted by a *filled rectangle*. (a) Transposition is initiated by an enzyme complex that causes two opposing single-strand cleavages separated by 3-4, 5, 9, or 11-12 bp, depending upon the transposable unit. (b) Subsequently, a second enzymatic component recognizes, cleaves, and transfers one end of the transposable segment to the recipient molecule, where it is ligated to a free 5'-end (see *asterisk*). As the recipient molecule is opened to accomodate the displaced strand, complementary strand synthesis occurs on the lower strand from the free 3'-end (see *dashed line*). (c) Complementary strand synthesis continues in the recipient molecule until the entire transposable element has been copied, at which point the bottom recipient strand is ligated *(asterisk)*. Because of lack of a primer, no complementary strand synthesis has occurred in the donor molecule. (d) Thus, the displaced strand is retransferred to the donor molecule and complementary strand synthesis occurs on the top recipient strand from the free 3'-end. (e) Following ligation of the ends of the transposable segment to the adjacent chromosomal DNA, the donor transposable unit is completely conserved. For more details of this proposed model, see Grindley and Sherratt (1979)

Sherratt 1979), Shapiro (1979) has proposed a different approach to the transposition event. According to this scheme, a circular donor molecule containing one copy of a transposable unit is co-integrated into the recipient molecule. Subsequently, the donor molecule is deleted along with a hybrid copy of the transposable unit while leaving another copy of the transposed segment flanked by duplicated recipient sequences. The details of this proposal are given in Fig. 18 (Shapiro 1979). A significant facet of this model, cointegrate formation/replicon fusion, is a normal intermediate structure of the transposition process between interacting circular molecules and can be observed whenever the final reciprocal exchange event is blocked. Furthermore, this model can produce deletion and inversion events during transposition if the donor and target DNA regions are on the same molecule (for details see Shapiro 1979). More recently, a very similar transposition model was proposed by Arthur and Sherratt (1979).

3) Other Aspects of Transposition. Do transposable elements exist, as transposition intermediates, in the autonomous, nonreplicating circular state? By biochemically splicing a known small replicon into the central sequences of a transposable element, Cohen et al. (1979) have isolated a self-replicating transposition "intermediate". Although one wonders if this structure is merely a product of precise excision, it should prove valuable in further defining the transposition process. Similarly, further study of the recently isolated single-strand phages carrying transposons may aid our understanding of transposition (Nomura et al. 1978; Ray and Kook 1978).

The actual recognition sites in transposons or in recipient DNA for the transposition-enzyme complex have not been deciphered, but it is noteworthy that the ends of transposable elements are palindromic and A+T rich. Palindromic DNA sequences are known to be protein interaction sites for a variety of enzymes and repressor molecules; and A+T rich regions are susceptible to "localized denaturation", perhaps prior to cleavage (see Vogel 1977). It seems likely that the inverted repeat sequences within and at the ends of the termini of transposable segments serve as recognition sequences. Comparison among the ends of various transposable elements has revealed that the ends of Tn*A* and Tn*3* (both of which produce 5 bp direct repetitions; M. Guyer and N. Grindley, personal communication) or the ends of Tn*10*, Tn*9*/IS*1*, and Tn*903* (all of which produce 9 bp direct repeats; Kleckner 1979) share significant segments of homology. In addition, Kleckner (1979) has reported very limited evidence for sequence homology between a site internal to Tn*10* and a region in the recipient molecule, but some distance from the insertion site, that helps align the incoming Tn*10* element with respect to its insertion site. Alternatively, and more likely, the non-random clustering of transposon insertion sites suggests that the enzyme component that makes the 3-4, 5, 9, or 11-12 bp staggered cut in the recipient sequence is similar to type I restriction endonucleases, which are known to cleave DNA at a distance from the actual recognition site (Rosamond et al. 1979). Finally, one wonders if the apparent regulation of transposition frequency is not a result of modification of the recipient DNA recognition sequences.

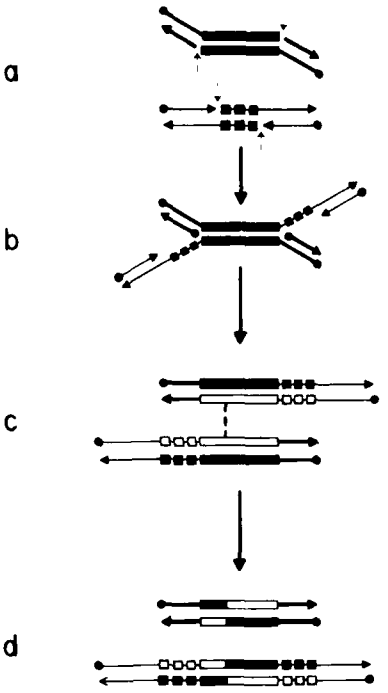

Fig. 18. Fusion model of transposition. Double-stranded donor *(thick lines)* and recipient *(thin lines)* DNA regions are shown horizontally. Only the interacting portions of these DNA regions are illustrated; they may exist on the same or different molecules. The transposable DNA unit is represented by a *rectangle*, while the target site is depicted by *small boxes*. (a) Transposition is initiated by four single-strand cleavages (shown by small *vertical arrows*), one at each end of the target site and transposable DNA segment. The two pairs of cleavages must have opposite polarities. *Arrowheads* and *tips* on these strands represent 3'-OH and 5'-PO_4 ends, respectively. (b) Subsequently, the donor and recipient strands are joined in a chi-shaped structure by ligation at points marked by small arrows. Replication from the free 3'-end is thought to produce two duplex DNA regions, each carrying a semi-conservatively replicated transposable unit, as shown in (c). Newly synthesized DNA is represented by *open blocks* and *rectangles*. If both initial interacting DNA regions represented different circular molecules, this step would result in a figure eight or replicon fusion structure. A final reciprocal exchange is proposed to take place between the repeated transposable units (see *vertical dashed line*), generating separate donor and recipient molecules, each carrying one hybrid copy of the transposable unit. (d) In addition, the recipient molecule contains direct repeats of the target sequence flanking the newly inserted DNA segment. For details see Shapiro (1979)

V. Bacterial System(s) for *recA*-independent Recombination

The spontaneous occurrence of chromosomal deletion or duplication events in the absence of known general recombination systems has been recognized for some years (reviewed by Franklin 1971). The discovery of transposable DNA units and our newly acquired knowledge of how Mu phage and IS elements create chromosomal rearrangements can explain the formation of virtually any chromosomal reshuffling. Despite these remarkable findings, there are still many spontaneous deletion and duplication events that do not appear to involve either general recombination or any of the known specialized recombination systems. The recent advances in nucleotide sequencing techniques have allowed for a long-awaited examination of the nucleotide sequence relationships among the ends of these aberrant duplication and deletion mutations.

Evidence obtained from nucleotide sequence studies of *lacI* gene mutants indicates that DNA segments located between identically oriented repeats of 5 or 8 bp can be deleted. The deletion always removes one repeat sequence and all of the intervening sequences. Also, duplication of a DNA segment that lies between small, identically oriented repeats has been observed (see Farabaugh et al. 1978). However, it is not known if these sequence-specific chromosomal alterations occur *recA*-independently. In a separate study, nearly precise excision of Tn*10*, which occurs in the absence of Rec ability, has been observed to result apparently from recombination between 24 bp repeat sequences that are located at each Tn*10* terminus (Ross et al. 1979b). Although all of these rearrangements could have occurred during replication or repair by mispairing events, one can also speculate that an uncharacterized bacterial system(s) for genetic exchanges at short, repeat DNA sequences might be responsible. As mentioned previously, precise excision by cleavage at the specific termini of a transposable element or Mu prophage and subsequent closure of the chromosome would leave one extra 3-4, 5, 9, or 11-12 bp copy of the recipient site sequence. Perhaps some system like that discussed above is involved in the deletion of Tn or IS units so that one copy of the repeated recipient site sequence and the entire intervening sequence (i.e., the Tn or IS unit) are deleted. Such a system is not entirely without precedent. The integration and excision of lambda phage occurs between 15 bp common core repeat sequences, one each in *attP* and *attB*. The sequences surrounding the core region are involved in the specificity and requirements of the reaction. Likewise, the chromosomal sequences surrounding any repeat sequence on a molecule may affect its reactivity/involvement in recombinational exchanges. Though seemingly logical, these suggestions are very speculative.

An unusual recombination pathway in *E. coli* has recently been detected during the study of recombination between different, genetically marked λ phage. Neither host Rec nor λ Red, Int or Der pathways of recombination appear to be responsible. In *recA* cells, these recombination events occur at a frequency of ~10% of that seen in Rec^+ hosts and appear to involve exchanges be-

tween homologous DNA regions. Furthermore, these events require RNA polymerase and probably active transcription; this has been termed the "Rpo pathway". It has been proposed that some local change (e.g., unwinding) of DNA structure caused by transcription is required for this process (Ikeda and Kobayashi 1977). It is not yet known if such a process is involved in specialized transposition or the precise excision events discussed above. It is interesting to note, however, that the Ohtsubos have found sequence homolgy between the ends of IS1 and known genetic promoters (i.e., RNA polymerase binding sites) and have proposed that RNA polymerase and perhaps the Rpo pathway are involved in Tn or IS unit transposition and/or precise excision (Ohtsubo and Ohtsubo 1978).

D. Effect of Transposable Elements and Viruses on Bacterial Evolution

Transposable genetic elements appear to be normal constituents of bacterial, plasmid, and phage chromosomes. Conjugative plasmids allow for the rapid intercellular dissemination of genetic information and have been identified in many diverse bacterial species (Reanney 1976; Kopecko et al. 1979). Considering the fact that conjugally promiscuous plasmids can transfer between quite different bacterial genera, it appears reasonable to suggest that transposable genetic elements exist universally in bacteria.

Temperate viruses and transposable elements (IS, Tn units) cause mutations and mediate macro-evolutionary chromosomal rearrangements. In addition, some transposable elements are involved in the regulation of gene expression. Specific examples of how transposable DNA units affect bacterial evolution have been given in the previous sections and have been extensively reviewed (Cohen 1976; Cohen and Kopecko 1976; Starlinger and Saedler 1976; Bukhari et al. 1977; Kleckner 1977; Starlinger 1977; Schwesinger 1977). This section is intended to focus on several specific points that have not been discussed elsewhere.

1. The Chromosome - Constancy in the Face of Change

IS1 through IS5 and γδ, from data presented above, exist as 8, 7, 5, 1, 1, and 4 copies per genome, respectively, which represents 1 % of the 4.6 x 10^6 K-12 chromosome. In fact, many segments of the 4.6 x 10^6 K-12 chromosome that are bordered by inverted repeat DNA segments of 750 bp or larger have been visualized in the electron microscope (Chow 1977; Ohtsubo and Ohtsubo 1977). Since the chromosome is circular, all genes lie between one or more sets of inversely repeated DNA segments. The known locations of IS elements on the 4.6 x 10^6 K-12 chromosome and the F plasmid are shown in Figs. 19 and 20. As discussed below, recombination between IS elements carried on plasmids (e.g., the F plasmid) and chromosomally borne IS units results in the chromosomal integration of the plasmid (Davidson et al. 1974).

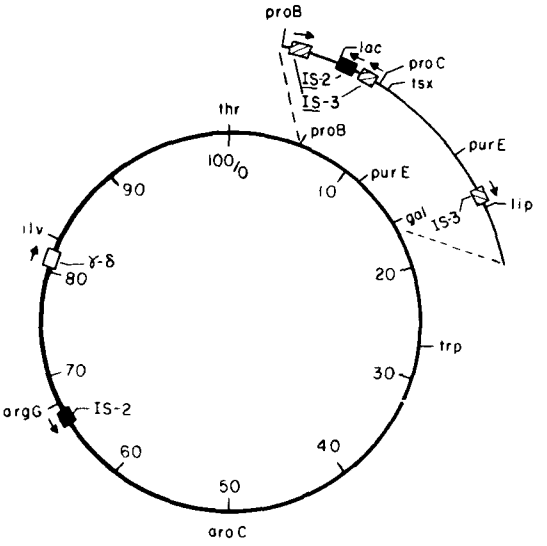

Fig. 19. Insertion sequences act as recombinational "hotspots" for Hfr formation. The circular linkage map of *E. coli* K-12 is schematically divided into 10 min length segments and the chromosomal locations of various genes and insertion sequences are shown. The genetic markers listed include those that affect the synthesis of threonine (*thr*), proline (*pro B*, *pro C*), adenine (*pur E*), tryptophan (*trp*), chorismic acid (*aro C*), arginine (*arg G*), and isoleucine-valine (*ilv*); those that affect utilization of lactose (*lac*) or galactose (*gal*); and those that affect the requirement for lipoate (*lip*) or the resistance to phage T6 (*tsx*) (see Bachmann et al. 1976). Through an intensive study of the molecular relationships among F and several F' plasmids, N. Davidson and co-workers (1974) have deduced the identities and physical orientations of the insertion sequence elements that are actively involved in Hfr formation (indicated by [illegible] in the figure) at six different locations on the *E. coli* genome. Although the *E. coli* K-12 chromosome was estimated, by DNA-DNA hybridization studies, to carry seven copies of IS', the locations of only two such sequences are known. To create an Hfr strain the autonomous F plasmid apparently integrates into the chromosome after complementary pairing between an insertion sequence region in the F plasmid and a homologous sequence in the chromosome. Thus, Hfr polarity would be a direct consequence of the orientations of the homologous sequences on each parental molecule. Twenty-seven different Hfr strains are thought to have been formed by F integration at one of the sequences mapped above. It is likely that other known Hfr strains were constructed by F integration at other insertion sequences located on the chromosome which have not yet been mapped.

The frequency of spontaneously formed duplication in the *E. coli* chromosome has been reported to be 10^{-4}-10^{-7}, while spontaneous deletion formation has been estimated to be 10^{-6}-10^{-9}, similar to point mutations (see Starlinger 1977). Non-randomness of end points has been noted for both duplication (Starlinger 1977) and

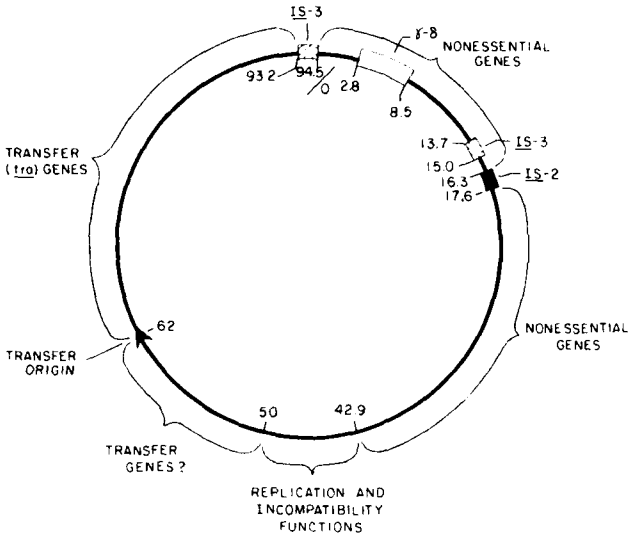

Fig. 20. Structural map of the F (sex) plasmid. In this scaled map, the locations of various functions, constructed from published data, are physically positioned through the use of a kilobase (kb) coordinate mapping system devised by N. Davidson and co-workers (1974). The map order of 12 genes that determine the necessary functions for conjugal transfer (*tra*) plus two associated regulatory genes is known, and these genes occupy the last one-third of the total 94.5 kb pair length of the F plasmid. The origin of conjugal transfer has recently been mapped accurately at kilobase coordinate 62 and the region between kilobase coordinates 50 and 62 may contain other transfer-associated functions (M. Guyer, personal communication). By the use of restriction enzyme procedures, construction and study of large letion mutants of F has led to the conclusion that all functions necessary for self-replication and incompatibility (a locus responsible for the inability of two similar plasmids to coexist within the same cell) are encoded on a small 7.1 kb long DNA segment. The regions labeled as non-essential genes have been genetically deleted without observed consequences on either F self-replication or transfer ability. Additionally, locations are given for IS^{3}, IS^{2}, and the gamma-delta sequences, all of which are separate integration sites for Hfr formation, and which also participate in F plasmid recombinational rearrangements

deletion mutations (Farabaugh et al. 1978). Spontaneous transposition and inversion events have also been observed in [illegible] and their instability suggests the presence of terminal repeat sequences which subsequently undergo [illegible]-dependent reversal of the original event (Starlinger 1977). Although some of these events would likely be IS-mediated, recent evidence indicates that deletions and duplications can occur via a bacterial [illegible]-independent recombination system(s) which recognizes similarly oriented, short repeat DNA sequences (discussed above). In addition, other as yet uncharacterized recombination systems are

probably also involved. Much ado has been made about the Rec-independence of specialized recombination events and it should be emphasized that Rec-dependent recombination between these transposable elements (i.e., mobile regions of homology) probably is more important evolutionarily.

It is obvious that chromosomal rearrangements occur quite often in bacteria, but why bacteria apparently remain genetically stable is not so obvious. Evidently, most genetic alterations, even those affecting only a few nucleotides, are not evolutionarily advantageous. Perhaps each small region of the chromosome has been and is being constantly exposed to different surrounding sequences, always striving for the most stable arrangement for that particular environment. Thus, when mutations occur but the environment remains essentially unchanged, the mutations would be selected against. As the environment and, consequently, cellular requirements change, various other chromosomal rearrangements are selected for and the chromosome would remain in a quasi-stable state while the individual nucleotide regions seek their most stable interrelationships for the new environment. Observations made with cloned eukaryotic DNA in E. coli cells (Cohen et al. 1978) or results of analyses of single plasmids isolated from different bacterial hosts (Causey and Brown 1978) support this conjecture.

II. Plasmids - Recombinational Assemblages of Transposable Units

Early electron microscope heteroduplex analyses of the nucleotide sequence relationships among F and related R plasmids indicated that these plasmids are largely homologous. However, areas of homology among these molecules terminated at identical points in several different plasmids, hinting at the modular nature of plasmid evolution (Sharp et al. 1973; Cohen and Kopecko 1976). Cataloging of the properties of many transposons, detected on plasmids obtained from a large variety of different bacteria, has clearly demonstrated the fact that plasmids are evolving through a process of exchange of discrete transposable units. For instance, knowing the location of several transposons on the R1 plasmid, Kopecko et al. (1976) deduced from the earlier data of Sharp et al. (1973) that the R100, R6, and R1 plasmids, though obtained from bacteria originally isolated in Japan, Germany, and England, respectively, carry some of the same transposable elements (see Fig. 21). Moreover, the finding that the shared transposons were located at the same location in each R plasmid further suggested that these plasmids have evolved from a common ancestral plasmid. In another example, Tn1, Tn3, Tn4, Tn5, Tn7, and Tn10 are members of a class of transposons, collectively called TnA elements, that encode ampicillin resistance. These highly homologous TnA elements, detected initially on different plasmids isolated from Escherichia coli or S. typhimurium, have more recently been detected in plasmids from Haemophilus and Neisseria (Falkow et al. 1977). Thus, transposons have played a major role in the dissemination of drug resistance genes and other medically relevant determinants (e.g., enterotoxin or K88 antigen synthesis) among bacteria.

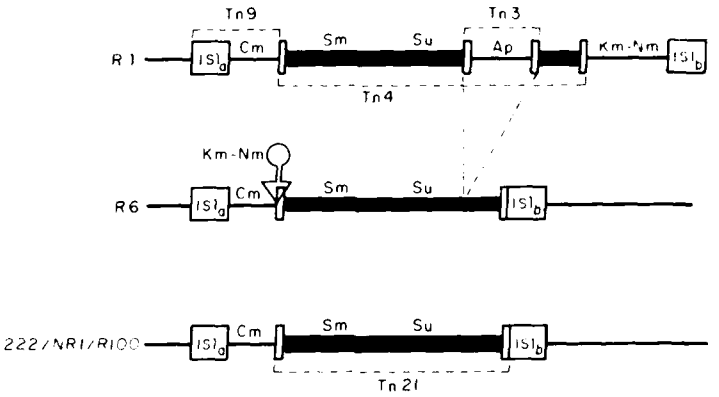

Fig. 21. Relationship of r-determinant regions on R1, R6, and 222/NR1/R100 plasmids. Only the r-determinant region of these plasmids is depicted; direct repeats of IS1 were found to bracket the r-det region (Hu et al. 1975; Ptashne and Cohen 1975). R1, R6 and 222/NR1/R100 are plasmids that were obtained from different bacteria isolated initially in England, Germany, and Japan, respectively. The same transposable sequence encoding chloramphenicol resistance (Cm) is located in all three plasmids as well as the larger transposon encoding streptomycin (Sm) and sulfonamide (Su) resistance. However, in the R1 plasmid the Tn3 (Ap) element is inserted within the Sm, Su transposon and now comprises the composite transposable element, Tn9. The R1 plasmid also carries an additional segment which encodes resistance to kanamycin-neomycin (Km-Nm) and which is deleted spontaneously at a high frequency, but has not been shown to transpose (Kopecko and Cohen 1975; Kopecko et al. 1976). Finally, the R6 plasmid carries an inserted transposable kanamycin/neomycin resistance gene segment located between the Cm and the Sm, Su transposons (see Kopecko et al. 1976, 1978)

Susskind and Botstein (1978) recently reviewed considerable data which indicated that P22 and the lambdoid viruses share some identical functional DNA segments. These authors suggest that these viruses are comprised of a set of interchangeable modular units, but the mechanism of interchange is unknown.

Many plasmids carry, in addition to transposons, IS elements which allow for chromosomal integration of plasmids. For example, during Hfr formation the F plasmid, shown in Figure 20, integrates into the chromosome via an homologous IS segment shared with the chromosome (Fig. 19; Davidson et al. 1974). Plasmid-borne IS units are also used to amplify genes located between two similar IS elements (Cohen 1976; Yagi and Clewell 1977; Schmitt et al. 1979a).

III. Evolution of Transposable Elements

From an evolutionary viewpoint it seems instructive to mention that although IS elements can transpose as independent units, it appears that two nearby, identical IS elements flanking any

sequence can form a transposon (MacHattie and Shapiro 1978; So et al. 1979; M. Guyer and J.L. Rosner, personal communication). Considering the recent observation that the *his-gnd* region of the *E. coli* chromosome is a transposon, is every gene on the chromosome located on a transposable segment at one time or another when bracketed by IS elements?

There does appear to be a hierarchy of transposable elements beginning with IS units and ending with the most complex unit, Mu phage. It is possible that transposons containing long inverted repeat termini (e.g., Tn*10*) which are not susceptible to *recA*-dependent deletion, are more highly evolved than Tn*9*, for instance, which is flanked by direct repeats of IS*1*. Furthermore, Tn units encoding functions necessary for their own transposition would seem more advanced than units that use host-encoded transposition machinery.

The origins of transposons are unknown, but the similarity of the terminal sequence, as determined by sequence analysis and discussed earlier, of some transposable elements would suggest the existence of four evolutionarily separate classes of transposons (those with 3-4, 5, 9, or 11-12 bp repeats). The minimal length of a transposable segment is not known but one might assume that it could be as short as two adjacent recognition/cleavage sites, which might be as short as 4-8 bp each. However, this seems unlikely because one would then expect there to be innumerable IS units and, instead, only a few classes have been detected.

E. Use of Transposable Elements as Experimental Tools

Two recent extensive and masterfully composed reports on the practical in vivo employment of transposons and Mu phage in experimental genetic manipulations are available (Faelen and Toussaint 1976; Kleckner et al. 1977). However, a brief listing of the experimental uses of Tn elements has been included here for general information and to stimulate interest in these experimental tools. The ease of selection for a drug resistance phenotype, the ability of transposons to insert at many chromosomal sites at a relatively high frequency and, in addition, to generate various chromosomal rearrangements make Tn elements very useful in the laboratory. Transposons can be introduced into a bacterial cell via infection with defective transducing phages carrying a Tn element (Kleckner et al. 1975), or by conjugation or transformation of plasmid vectors containing Tn's. These vectors can be eliminated by a variety of procedures, e.g., conditionally lethal mutations or conditions (Kretschmer and Cohen 1977). Mutations in virtually any chromosomal site can be obtained by simultaneous selection for the transposon phenotype (e.g., tetracycline resistance) and loss of the vector. Subsequently, isolated colonies can be replica-plated on appropriate media to obtain the desired mutants. Transposon-induced mutations can be polar, allowing for the location of genes in an

operon, as well as for genetic mapping of mutants with phenotypes that are not scorable (Kleckner et al. 1978). Furthermore, these remarkable Tn-induced mutations are revertible so that the original phenotype can be restored.

Tn units, inserted into sites immediately adjacent to the trait(s) of interest, can be used to manipulate a particular trait by chromosomal duplication or deletion, as described below. In addition, a non-scorable trait can be molecularly cloned by selecting for an easily identifiable adjacent drug resistance transposon. Transposons can also be used in genetic engineering to generate known restriction endonuclease cleavage sites in specific chromosomal regions. Furthermore, inserted Tn elements can mediate deletions from the element outward to various chromosomal sites. Alternatively, two identical Tn elements inserted at nearby chromosomal sites can generate specific deletions of the interposed sequences. By similar techniques, Tn*10* has been used to generate chromosomal duplications with predetermined end points (Kleckner et al. 1977) and probably could be used to promote inversions of specific chromosomal genes. It should be emphasized that fusion of nearby, but unrelated DNA sequences (e.g., two different operons) can be constructed by deleting the sequences located between Tn units inserted within each operon, followed by selection for precise excision of the remaining Tn element.

As noted in previous sections, Tn units can promote the random chromosomal integration of both plasmids and phage, a condition which is conducive to the subsequent formation of a variety of novel specialized transducing phages and plasmids carrying various chromosomal segments (e.g., F-prime plasmids). Moreover, recombination between a Tn unit located on a conjugative plasmid, like F, and an identical Tn unit inserted at a known site on the bacterial chromosome will lead to the construction of Hfr strains with predetermined transfer origins and orientations. Transposition of Tn elements to plasmids that are phenotypically cryptic or not easily scorable offers new possibilities for the manipulation and study of these elements. In addition to transferring traits of interest through the above manipulations, it appears that one can construct new transposable elements by inserting known IS or Tn units to either side of the DNA segment of interest (McHattie and Shapiro 1978; Shapiro and MacHattie 1979; M. Guyer and J.L. Rosner, personal communication) or by the molecular cloning of a DNA segment into the middle of a characterized transposon (Goebel et al. 1977; Heffron et al. 1978; J. Manis and B. Kline, personal communication). The usefulness of Tn units as experimental tools is not limited to *E. coli* by any means, as Tn elements can be transferred to a wide variety of bacterial genera by conjugally promiscuous plasmids such as RP4. In addition, the Tn elements in gram-positive bacteria should prove to be just as useful experimentally. Finally, one should be aware that Mu phage can mediate all of the events listed above and may be useful under conditions in which Tn units cannot be employed.

F. Specialized Recombination in Eukaryotic Cells: A Prologue

A rapidly increasing body of evidence indicates that specialized recombination systems are not unique to prokaryotes, but rather are found in a wide range of eukaryotic organisms as well (see Bukhari et al. 1977). From the results of classic genetic studies of maize, McClintock (1957, 1965) has described distinct transposable genetic "controlling elements" that are capable of affecting the expression of various genes. Like IS segments, insertion of a controlling element into a gene can cause inactivation of that gene; restoration of gene activity occurs following excision of the controlling element. In addition, major chromosomal rearrangements (inversion, deletions, duplications) are often found in association with loci carrying these elements. Recently, Nevers and Saedler (1977) have composed an excellent summary of controlling elements in maize and, based on known properties of bacterial IS units, offered an elegantly simple model to explain their behavior (see also Peterson 1977). Furthermore, considerable genetic and cytological data obtained with *Drosophila* suggest the presence of transposable "IS-like" elements that are capable of causing mutations and site-specific deletions (Green 1977). In addition to mediating host chromosomal integration of various eukaryotic viruses, it seems reasonable to assume that specialized recombination systems are involved in transposition of the controlling elements in maize and the IS-like mutants in *Drosophila*, as well as in mediating various chromosomal reshufflings associated with these mobile DNA units.

Several examples of potential specialized recombination systems have been observed in yeast. The alternating and exclusive expression of one of two mating types in *Saccharomyces cerevisiae* has been hypothesized to occur via exchange of specific DNA segments (the cassette model; Hicks et al. 1977). A similar system for mating-type interconversion seemingly exists for *Schizosaccharomyces pombe* (Egel 1977). With the use of relatively new molecular cloning systems, Cameron et al. (1979) recently have physically identified transposon-like elements in the DNA of *S. cerevisiae*. One element, TY1, is 5.6 kb in length and is flanked by a 0.25 kb direct repeat, termed delta. Hybridization studies show that TY1 is present as 35 copies per haploid genome (i.e., 2% of the total haploid DNA content), whereas delta, which is not always associated with TY1, exists as 100 copies per haploid cell. Furthermore, both TY1 transposition and linked chromosomal alterations have been observed. Although speculative, middle repetitive DNA, like TY1, which may be involved in gene regulation in eukaryotes, could be transposed via specialized recombination processes.

Finally, numerous eukaryotic genes have been identified which are interrupted within the coding regions by intervening sequences. These intervening sequences are found in the primary transcript of the gene, but not in the functional mRNA, i.e., they are spliced out and the resulting ends of the RNA molecule are rejoined (Darnell 1978; Knapp et al. 1978; Tilghman et al.

1978). Are there specialized recombination systems for exchange between RNA molecules?

Acknowledgements. I am indebted to my wife, Patricia Guerry-Kopecko, who helped with the editing, writing, and final compilation of material and without whose help and encouragement I could not have written this review. I appreciate the helpful advice of P. Barth, A. Campbell, R. Deonier, N. Grindley, M. Guyer, M. Howe, A. Puhler, H. Saedler, F. Stahl, W. Szybalski and A. Toussaint and thank the numerous co-workers who sent me reprints and preprints of their work, especially P. Bennett, D. Botstein, S. Cohen, M. Howe, N. Kleckner, H. Nash, E. Ohtsubo, H. Saedler, R. Schmitt, W. Szybalski, and A. Toussaint. Finally, I thank L. Baron and J. Wohlhieter for their patience and encouragement and for reviewing the manuscript.

This review was completed in July 1979.

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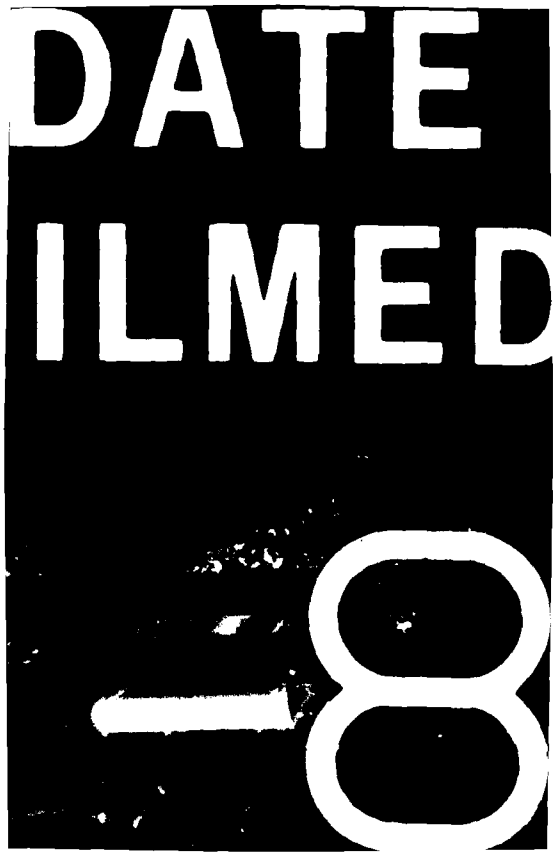
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